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It is characteristic of Science and Progress that they continually open new fields to our vision.—Pasteur

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FOREWORD

THE GENESIS OF A NEW SCIENCE,—BACTERIOLOGY

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"*Die Bakteriologie ist ein Kind der jüngsten Zeit.*"—FRAENKEL, 1886.

Sciences are not made but born, and lineage often sheds light upon development. It was an acute observation of the late C. S. Peirce that some of the most fruitful of modern sciences have been bred by the crossing of older ones. Mathematical astronomy, physical chemistry, physiological psychology, astrophysics are examples, and the same thing is true of the applied sciences, as witness electrical, chemical and sanitary engineering; bio-chemistry; electro-chemistry.

Bacteriology is the offspring of microscopical science hybridized with the art of bacterial cultivation,—in other words, of microscopy and bacterial horticulture. The compound microscope was invented and bacteria and other micro-organisms were observed in the seventeenth century but no great progress was made in our knowledge of microbes—with the single exception of yeast—until methods for their selective cultivation or breeding similar to those long practised in agriculture and horticulture were discovered and introduced by Pasteur, Lister and Koch. In his studies in zymology and his long and arduous battle against spontaneous generation, Pasteur became proficient in "sterilizing" nutrient liquid soils or "substrata" which he afterwards planted or "inoculated" with "traces" of micro-organisms. These traces after incubation and multiplication produced overgrowths if not pure cultures of particular kinds of micro-organisms, in manageable quantities sufficient for reasonably thorough examination. In this way, Pasteur

was able to magnify microscopic into macroscopic characteristics, and for the first time made it practicable to differentiate and classify bacteria with some accuracy. It should also be remembered that Pasteur's disciple and follower, Joseph Lister, made improvements in the method of pure culture by "dilution" during his studies upon milk and the lactic fermentation.

Pasteur, very early in his work, had insisted upon the indispensability of the microscope in all investigations of yeasts and other microbes, as well as in fermentations, putrefactions and diseases (of wine and beer), which they produce, and only those who have taken the trouble to read the preposterous paper in which Liebig, the most eminent chemist and fermentation expert of his day, ridicules the use of the microscope—a paper which Huxley has rightly pronounced the most surprising that ever appeared in a sober scientific journal—can appreciate the immense service done by Pasteur in developing the microscope as an instrument of research. It was his insistence upon the use of the microscope superadded to a rigid and refined technique all along the line which enabled him to win one of the hardest fought and most important scientific battles of the nineteenth century, namely, that on behalf of biogenesis.

Pasteur is thus at once the pioneer and the founder of that wonderful science of which the present new JOURNAL is to stand as an American exponent.

But it is very doubtful if bacteriology would ever have attained even a tithe of its present development and importance if the methods of Pasteur and Lister had not been supplemented and largely displaced by those of Robert Koch, who is at once the protagonist of the new science and the architect of that imposing superstructure now known as bacteriology, built chiefly since 1881 by Koch himself and his pupils upon the foundations laid by Pasteur.

In his earlier work, Koch employed substantially the methods of liquid culture of Pasteur and Lister, but before long he vastly improved upon these cultures by thickening them with gelatine or agar—a step to which he was led through the use of potato an opaque medium for which it was obviously desirable to sub-

stitute something transparent. Brefeld and other botanists had already used gelatine, for "gelatine was first employed by Vittadini in 1852 in the culture of microscopic Fungi and has been frequently used since that time, especially by Brefeld. Klebs more recently, in 1873, recommends it especially for the cultivation of bacteria." (DeBary, 1886).

It was in 1881 that the gelatine-tube culture method and in 1883 that the gelatine-plate culture method were introduced by Koch—his first great work, namely, that upon anthrax, and also that on typhoid fever, having been done before this time, and his well known "rules," "postulates" or "laws" having also been laid down before his perfection, if not before his invention, of the solid-culture method.

The word "bacteriology" had appeared before 1886 but the subject had no existence anywhere much before that time and very little for a year or two later. In the United States, bacteriology was hardly heard of before 1885 but by 1890 it had become well known. Unfortunately, neither Great Britain nor America can make any claim to the earliest work. The terms "sterilization" and "cultivation," in our modern sense, and the word "microbe," were introduced by Pasteur and his school. The terms "pure culture," "colony," "gelatine," "agar," the use of the oil immersion objective, and the art of dyeing microscopic specimens of bacteria, come from Koch and the German school. Bacteriology is now, however, very widely cultivated both in Great Britain and the United States, and it is fitting that a JOURNAL OF BACTERIOLOGY should be published in the English language in honor of the thirty or more years of service which one of the broadest and most fundamental of the biological sciences may now claim.

Because of the intensely practical bearings of bacteriology upon medicine, and especially because of the marvellous surgical and pathological discoveries which were the first, and must ever remain the greatest, fruits of bacteriology, its botanical, agricultural, sanitary, industrial, household and economic importance were at first obscured and neglected. But of recent years these have rapidly become clear and even conspicuous, and today

hardly any field of the vast domain of the parent science, biology, is more esteemed or more cultivated than is bacteriology. Nor is this strange for, thanks to the microscope and the methods of bacteriology, the microscopic world, of which almost nothing was known when Pasteur began his researches, stands before us today in a revelation of extent, variety, magnitude and interest second only in importance and impressiveness to that other and distant world which has been revealed by the telescope and the methods of astronomy. The region of the "infinitely little" seems, however, even more directly and intimately connected with our everyday life than that remoter world. The microscopic world, indeed, lacks the majesty and grandeur of that telescopic world which lies so far beyond our reach and overawes us with sheer distance, heat, light and immensity. But the revelations of the microscope and the lessons of bacteriology have so direct, so intimate, and so fateful association with almost every aspect of the conduct of our daily and personal life—with food and drink, with health and disease, with life and death even—that they gain in intimacy what they lose in grandeur.

Bacteriology must henceforward be recognized as a broad and fundamental branch of science, coördinate with, rather than subordinate to, the other grand divisions of biology such as medicine, agriculture, zoölogy and botany. It is today of immense theoretical and practical importance, and it bids fair to become vastly greater and more important tomorrow. Hence the obvious desirability of a JOURNAL which shall cover the whole field and be devoted to the subject in its broadest aspects.

The time has forever gone by when bacteriology can be regarded merely, or even chiefly, as the handmaid of medicine or pathology. It is no less the servant of agriculture, of industry, of sanitation and of household life. It is already important in sanitary engineering, and indispensable in the arts of food production and food conservation. In its further differentiation and development the present JOURNAL should be a powerful factor. May the event justify both our hope and our expectation.

Massachusetts Institute of Technology
Boston, February, 1916

THE PEDAGOGICS OF BACTERIOLOGY[1]

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I propose to consider the subject of teaching bacteriology from several standpoints, and especially the place of bacteriology in scientific education and in medical education.

The students who, in the past, have demanded a knowledge of bacteriology as a part of their instruction in general biology have been much fewer than the number that should be seeking this knowledge; in fact the demand has been really insignificant in comparison with the importance of the study. An explanation for the neglect of bacteriology as a part of the general training of students of the biological sciences is difficult to find, but it is evident that the teachers have been to blame, chiefly because they have failed to emphasize the importance of the study from an educational standpoint. They have been interested, more in the practical application of a knowledge of bacteriology, than in the development of the educational importance of the subject.

The aim in modern education is to train the individual for usefulness. With the present crowded curricula in schools and colleges it is essential that the material presented for the training of students be selected with the greatest care. Each study should be carefully weighed in order to determine its educational value. It is necessary not only to select the subjects to be taught, but also to arrange the order in which they may be presented so as to obtain the greatest benefit from each.

In the modern organization of society the interests of different callings are so diverse as to call for general as well as special training. This fact is now recognized in the preliminary education

[1] Presidential address, Seventeenth Annual Meeting, Society of American Bacteriologists, Urbana, Ill., December 28, 1915.

demanded of those who expect to enter the various professions. There is a special need for broad general education in science for all persons who wish to be equipped for the most efficient service to mankind. The aim of education should be, not merely to give information, but to indicate how that information should be used, and only in so far as education aids in the promotion of the general welfare, does it meet the ideal. The extent of the training of each individual must depend upon his ability to receive and apply the knowledge which is being disseminated in educational institutions.

A particular science may be studied from two principal aspects, namely, the practical application of the knowledge gained to the solution of problems in a special field, and the educational value of a knowledge of the science in broadening one's concept of the various forces and agencies in nature.

The science of bacteriology has extended its ramifications in so many directions that its study has become of interest and direct value to the student in many fields. A knowledge of bacteriology enters in a prominent way into most of the activities of mankind, and for this reason it should receive much wider recognition as a subject for general educational training than it is receiving today. The educational value of the study of bacteriology has received recognition slowly and for a study of such immense practical importance it has been taken up, for its educational value, by a comparatively small number of students in our colleges and universities. Yet there are few subjects taught that touch upon so many phases of man's activities or so many of the conditions influencing his environment as does a course in bacteriology, and, it is safe to say, few other subjects can have greater educational value. No one can fully appreciate the relation of bacteria to many vital problems without having studied the subject at first hand. It is only by seeing the activities of the bacteria in the test tube, under diverse conditions, that one can gain an insight into their prominent place in many biological processes.

The relation of the bacteria to the nitrogen cycle in nature is most illuminating to the student. The function of the bacteria

in the decomposition of organic matter as they work over the useless constituents of dead plants and animals into forms in which they may be utilized as food by the higher plants is of the greatest importance in nature. The control and purposeful utilization of this same function of the bacteria in the preparation and preservation of food materials for man and animals, and the relation of the bacteria to water and sewage purification, are examples of the regulation of bacterial action for the economic and hygienic advantage of the human race. Of equal significance are the utilizations of the functions of the bacteria in agriculture, in domestic science and the industries; and of even greater importance are the methods of controlling the action of the bacteria in their relations to sanitary science and clinical medicine.

The earliest practical application of bacteriology was to the fermentation industries through the investigations of Louis Pasteur. This was soon followed by his pioneer work in diseases of animals, especially chicken cholera and anthrax. In this latter field Pasteur laid the foundations for our later work in immunology and protective inoculations while the studies of Robert Koch paved the way for the application of bacteriology to the solution of problems in the etiology of disease and in sanitary science.

The earliest demand for a knowledge of bacteriology came from the medical profession, concerning the activities of the pathogenic bacteria, and the first courses were given to graduates in medicine. These were followed later by courses for undergraduate students of medicine, of dentistry, and of veterinary medicine. The extension of our knowledge of the activities of bacteria in nature, in fields other than disease production, soon led to the development of courses for the sanitarian, the student of dairying, and the student of agriculture. In all these courses chief stress was laid upon the practical application of the knowledge gained to the solution of problems arising in these different fields. The concentration of endeavor and interest along such lines has yielded a great fund of knowledge which is now being utilized in enhancing the welfare of man.

In recent years there has been a slowly growing demand for a

knowledge of bacteriology on the part of students in biology, especially by seniors in arts and sciences, and by graduate students. The chief interest of this group of students is to gain a broader insight into the relations of the bacteria to many of the important problems of life. Some of these students are preparing to teach biology, and others are already teaching in high schools and colleges.

While in the earlier courses, offered to graduates and undergraduates in medicine, the subject matter presented was intended largely to facilitate the application of the knowledge gained to practical questions in medicine and sanitary science, the courses for students in the arts and sciences have taken on a somewhat different aspect. For these students it has been deemed preferable to lay greater stress upon the broad fundamental biological principles involved and much less emphasis upon the practical application of the knowledge. The students of chemistry and biology in the graduate school, and in the senior class of the course in arts and sciences, have greater interest in the general information obtainable from the study of bacteriology, than in the more intricate problems of infection and immunity, which are of primary interest to the medical student. For this reason the general course in bacteriology for science students should be developed so as to acquaint them with the relations of the bacteria to problems of food production and conservation and to problems in domestic and sanitary science.

Bacteriology can be studied with greatest profit by students in their junior and senior years in college, or by graduate students, after they have had a broad training in biology, chemistry, physics and the languages. The student of bacteriology should have had instruction in general botany and zoölogy, in plant and animal physiology, in general inorganic and organic chemistry as well as in elementary physics. With a knowledge of these subjects he will be in a position to understand something of the biological relation of the bacteria to the welfare of man and especially to the problems of sanitary science. The broadening of the preliminary education of the medical student so as to include chemistry, biology, physics and the modern languages has

made it possible to place the teaching of bacteriology to these students on a much higher plane than was formerly attainable. Their understanding of the far-reaching activities of the bacteria has thereby been greatly increased and their application of the knowledge gained, to the solution of the problems which confront them as practitioners of medicine, is already showing abundant fruit in the more intelligent attitude which medical men are assuming toward questions relating to the public health.

The student who takes up the study of bacteriology as a part of his education in the biological sciences should possess a preliminary training equal to that required of medical students. With this broader foundation it becomes possible for the teacher to present the subject in a more philosophical way, and the general training which the student receives is correspondingly made more beneficial.

The amount of instruction in bacteriology offered to science students must vary with the time available for the study and with the general and professional training which the individual student is seeking. The minimum course should be one of twelve hours a week for one semester and should be devoted to general bacteriology. After the student has acquired some knowledge of bacteriological technique and of the general characters of the bacteria, attention should be directed to the activities of the bacteria in decomposition, in fermentation, in water and sewage purification, in the dairy industries and in food production and preservation. For students who desire more profound knowledge along the various lines of general and applied bacteriology, more detailed courses should be arranged to meet their special needs, the course to be given depending in part on the application which the students desire to make of the knowledge they are seeking.

The best course of study in bacteriology for the student in biology or general science has not as yet been developed. For students beginning the study a combined lecture, laboratory, and seminar course seems to give satisfactory results. The lecture should be largely a part of the laboratory exercises and should consist in explanatory remarks preceding each new phase

of the subject that is taken up. It is desirable to give the student a brief explanation as to what he is expected to do or see and how he is to proceed in conducting the laboratory exercises. When the student has carried out a series of laboratory exercises the subject may be developed on a broader plane by a lecture emphasizing the importance of the observations made and their relation to other aspects of the study.

The almost infinite number of ways in which the bacteria and their activities react upon human life, especially in their relation to the production of disease in plants and animals, and their relation to the various industrial activities, particularly in food production and food preservation, give us inexhaustible material for study in the classroom. The knowledge which the student of bacteriology gains is of such great personal interest and importance that he is easily carried along, step by step, from simple observations to the most complex and vital phenomena of life.

The study of bacteriology serves unusually well for training the powers of observation and judgment. Every lesson is per se an object lesson and one in which the student is not only the observer, but, the demonstrator as well. Moreover the remarkable susceptibility of the bacteria to environmental influences will permit of each demonstration being modified in a variety of ways. This possibility of varying the demonstrations precludes the probability of a loss of interest on the part of the student.

It will be profitable to begin a course in general bacteriology with exercises in staining various types of bacteria. The student should record his results briefly and amplify the record with line drawings of the organisms and cultures studied. In this way he acquires some knowledge of the relative size, grouping, staining reactions, and rapidity of growth of the bacteria. The next step may be the isolation of bacteria in pure culture from mixtures and the cultivation of several species of these pure strains upon the common media employed for this purpose. In the systematic study of a culture the student may follow the general plan of description as contained in the Society card. This will acquaint him with the vocabulary generally employed in this work and will help him to recognize some of the ac-

tivities of the bacteria. After he has become familiar with bacteriological technique and the methods of studying individual cultures, he should be given pure cultures of the common types of bacteria. It is desirable that he should be able to recognize all the ordinary bacteria that may be encountered later in his work as contaminations so as to be able to avoid confusing them with other bacteria that may be of importance in the study that he is conducting. With the foregoing exercises as a foundation the student is prepared to take up the study of the bacteria in water, soil, air, milk, butter, cheese; in the different orifices of the body; and in the excretions from the body. In these studies it will be possible to observe merely a few of the more common bacteria encountered, but each phase of the subject can be amplified by lectures, assigned readings, and discussions in the seminar. In the foregoing studies special exercises may be arranged to enable the student to comprehend the relation of bacteria to decomposition, putrefaction, fermentation, nitrification, denitrification and nitrogen fixation, or these phenomena may be independently attacked after the more common bacterial flora in nature have been studied. If the latter course is pursued the relation of the bacteria to these processes should be taken up briefly as phases of the phenomena present themselves, while the detailed study of the phenomena is carried out later.

A general course in bacteriology is not complete unless the student is given at least a brief introduction to the relation of bacteria to the diseases of plants and animals. This study should include the methods of recognizing the causative agents of disease, the manner in which they produce disease, and the ways in which recovery from infection occurs. The student should also have an introduction to the bacteriological side of important problems in preventive medicine, especially the efficiency of disinfection by the use of chemicals, heat and light. The relation of bacteria to the purification of water and sewage, and to the preservation of milk, eggs, meat and vegetables should be developed by lectures, assigned readings and exercises in the laboratory.

The position of bacteriology in the curriculum in some medical schools is unsatisfactory, especially where bacteriology is taught to first year students. It is largely a waste of time to attempt to teach clinical bacteriology to a student who knows nothing about disease in general and is not expected to take up the study of clinical medicine until one or two years later. The difficulty can be overcome in large part by requiring that the student receive a course of instruction in general bacteriology as a part of his premedical training and then receive instruction in clinical bacteriology during the second semester of the second year, or the first semester of the third year of his medical course. In the present arrangement of the curriculum of the medical school, if bacteriology is taught entirely in the first year, the student has usually not completed the study of physiology nor has he, as a rule, begun to study pathology and clinical medicine. The anomalous position of bacteriology in the medical curriculum is probably due to the fact that those responsible for the condition fail to appreciate the broad biological relations of the science of bacteriology.

The student of clinical bacteriology who lacks a knowledge of physiology, of pathology, and of clinical medicine, suffers a serious handicap in appreciating the principles that underlie the pathogenic action of the bacteria and the reactions of the body to infection. The problems of infection and immunity have the most important relations to normal and abnormal conditions of the body and these relations cannot be fully comprehended without a knowledge of physiology and of pathology.

Many of the colleges that prepare students for the medical course could be equipped, without great expense, if not already prepared to do so, to give a course in elementary bacteriology in their biological departments through teachers of those departments who would develop the subject on a broad biological basis. With such a preliminary training in general bacteriology the medical student could then take up clinical bacteriology with much greater profit in the second or third year of his medical course because he could appreciate the relation of the subjects to their clinical application.

If general bacteriology is placed in the premedical course it will be necessary to lengthen that course to three years, at least. This would involve no hardship for those students taking a combined science and medical course in seven years, nor for the students entering a medical school that requires a college degree as a requisite for entrance to the medical course. This plan would also afford opportunity to extend the premedical course so as to include organic chemistry and a broader training in biology and the modern languages, as many students enter the medical school with insufficient preparation in these three subjects.

In the second half of the second year or the beginning of the third year of the medical course, the student should have completed his studies in physiology and have had a course in general pathology. He would then pursue his studies in clinical bacteriology with much greater intelligence and profit.

The course in bacteriology adapted to the needs of medical students should consist, at present, of preliminary work in the acquirement of technique, the ability to isolate and recognize individual species of bacteria, the study of the common saprophytic bacteria and their important functions in nature, especially their relation to decomposition, putrefaction and fermentation, and the utilization of the functions of the bacteria in the purification of water and sewage. As persons with a broad scientific training, graduates in medicine should have as deep an insight into all of the foregoing activities of the bacteria as it is possible to give them.

With this fundamental knowledge the medical student is in a position to comprehend more fully the relation of the bacteria to disease, and the various measures which are employed by sanitarians to combat and eradicate disease.

The more practical side of the training of medical students will deal with the recognition of the pathogenic bacteria, a knowledge of the effects which they produce in the body in causing disease, and the reactions of the body in overcoming the disease.

The extent to which the medical student should be trained in the various phases of clinical bacteriology cannot be stated

categorically, but it may be emphasized that the more detailed the laboratory studies in infection and immunity, the greater the assistance to the student in obtaining a grasp of the subject and hence the more intelligent the application which he will make of the knowledge obtained, to the problems in clinical medicine and therapeutics.

If the medical student were to receive instruction in general bacteriology in his premedical course, it would be possible to devote more time to clinical bacteriology and its application to the diagnosis, treatment and prevention of disease during his medical course. Colleges and universities should therefore be equipped to give courses in general and special bacteriology to students in the premedical, the arts and sciences, and the sanitary engineering courses, as well as to science students in the graduate school. Besides courses in general bacteriology, more advanced courses should be offered, especially in the bacteriology of water and sewage, in dairy bacteriology, in agricultural bacteriology, in domestic science bacteriology and in sanitary science bacteriology.

It is evident that if the knowledge to be gained through a course in general bacteriology were more widely diffused amongst persons in all walks of life, there would be far less credence given to the extravagant and false claims of the horde of quacks and faddists who are now preying upon an ignorant and credulous public. The light of truth alone can relieve us of the depredations of those who claim to practice those "isms" that have been raised up because of the general ignorance of mankind.

In order to further the development of bacteriology and to extend the teaching of the subject to students of the biological sciences, it would be desirable for this Society to organize a teaching section for the discussion of problems in the teaching of bacteriology at each annual session. Through the interchange of views and the discussion of the principles of teaching the subject, the science of bacteriology, as well as education in general, would reap great benefit.

FURTHER STUDIES ON BACTERIAL NUTRITION: THE UTILIZATION OF PROTEID AND NON-PROTEID NITROGEN

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The highly interesting observation of Bainbridge (1911) that certain aerobic and facultative anaerobic bacteria of the gelatin-liquefying and non-liquefying types are of themselves unable to initiate decomposition of purified native proteins has been fully corroborated by Sperry and Rettger (1915). The last-named authors have shown further that the putrefactive anaerobes *B. putrificus*, *B. oedematis* (*B. oedematis-maligni*, Zopf) and *B. Feseri* (*B. anthracis-symptomatici*, Kruse) are likewise devoid of this property; and that the vegetable protein edestin, like egg and serum albumin, does not undergo disintegration by direct bacterial action. It was but natural to assume, therefore, that the protein nitrogen cannot be utilized by bacteria unless it is first simplified and made available for cell nutrition through the action of a proteolytic enzyme, strong acid or alkali, or some other cleavage-producing agent.

Solutions of purified proteins were prepared by the methods now used in all biochemical laboratories and involving the crystallization of the proteins. The test media were usually the same as those employed by Bainbridge, and contained the following ingredients, besides the protein; sodium chloride 0.5 per cent, sodium sulphate 0.2 per cent, calcium chloride 0.1 per cent and acid potassium phosphate 0.1 per cent. The only possible source of nitrogen was the protein, except in certain check tests in which small amounts of peptone were employed. The solutions containing the purified proteins were sterilized by filtration through the laboratory Berkefeld.

The test media were inoculated from 24 hour slant agar cultures of the various organisms, with the special precaution of

introducing only a small number of the bacteria and as little extraneous matter as possible. The fate of the bacteria and of the proteins was determined in three ways; first, by the plate method of determining the numbers of cultivable bacteria at the beginning of the experiments and after varying intervals or periods of incubation; second, by noting any change in the appearance of the media; and finally by determining the amount of coagulable protein at different times, during the course of the experiments.

The results obtained by Sperry and Rettger (1915) were so definite and consistent as to leave no doubt as to their significance. It was assumed that the purified proteins resisted decomposition by direct bacterial action because of their original or unchanged condition as native proteins; hence, sterilization by heat was to be avoided, as heating at coagulation temperature undoubtedly causes changes in the protein molecule.

The present investigation is in part a continuation of the work of Sperry and Rettger on the action of bacteria on purified proteins. Instead, however, of studying the behavior of bacteria toward unchanged (unheated) proteins, the experiments were conducted on test media containing coagulated egg albumin as the only possible source of nitrogen. The investigation also included a study of the behavior of bacteria toward proteoses and peptones, and of so-called "bacterial autolysis."

I. THE BEHAVIOR OF BACTERIA TOWARD PURIFIED COAGULATED EGG ALBUMIN

The egg albumin was prepared by the method of Hopkins and Pinkus (1899). The test medium containing the albumin and inorganic salts was the same as that used by Bainbridge and by Sperry and Rettger, with the exception that the medium was sterilized by heat and hence contained *coagulated* albumin. The methods of inoculation, incubation and determination of results were the same as those described in the earlier paper from this laboratory, (Sperry and Rettger, 1915). Special attention was given to the enumeration of bacteria by the usual plate method

immediately after inoculation and after definite periods of incubation at 30°C. The results are given in the accompanying Tables I and II.

With very few exceptions, the results show that there was little multiplication of the bacteria with which the medium was inoculated. An increase of 100 per cent, or even 1000 per cent,

TABLE I

*Enumeration of bacteria in inoculated test medium containing heated (coagulated) egg albumin. Counts per cubic centimeter of medium**

ORGANISMS	IMMEDIATELY	24 HOURS	48 HOURS	72 HOURS	4-7 DAYS	2 WEEKS	3 WEEKS	4 WEEKS AFTER INOCULATION
Prot. vulgaris I	2,600	3,752	2,350			4,800		
Prot. vulgaris II....	1,150	4,450	5,700			XX		
Prot. vulgaris III...	146	3,900			2,800			1,775
Prot. vulgaris IV...	358	1,650		4,032	1,070	3,520	3,840	
Prot. vulgaris V....	800	3,680	3,136			4,990	3,700	
Prot. mirabilis.....	480			1,980		3,000	544	1,180
B. subtilis.....	1		1,470			XX		XX
B. prodigiosus.....	640		XX			XX		XX
Staph. aureus II....	1,400	2,500	2,150					
Staph. aureus II....	3,800	4,800	7,450					
Staph. aureus III...	50	2,100			1,700			406
Staph. aureus IV...	365	864		990	860	134	75	
Staph. aureus V....	204	800	865			84	620	
Staph. aureus VI...	150				640	160	89	53
B. coli I.....	17				2,000	1,200	600	675
B. coli II.....	352			2,300		2,000	260	350
B. coli III.....	1			3,800		4,700	6,100	7,200
B. typhi I.....	288		705			480		162
B. typhi II.....	165			191		768	832	

NOTE: XX indicates too many colonies on the agar plates to count.

* Dilutions of 1 : 10,000 were employed in these tests.

in the numbers of organisms would not be unexpected even in what may be termed a nitrogen-free medium which is constantly exposed to the atmosphere. In the tests with *B. prodigiosus*, *B. subtilis* and one of the *Proteus vulgaris* strains the numbers of colonies on the agar plates became too numerous to count. Furthermore, there were visible indications that the protein was undergoing disintegration. These results are, therefore, in strik-

ing contrast to the rest. The most plausible explanation of the proteolytic action in these tubes is that these organisms produced a very active proteolytic enzyme early in the course of their growth on the slant agar, so that sufficient enzyme was introduced into the test medium along with the bacteria to bring about cleavage of the albumin and thus prepare it for nitrogen assimilation by the bacteria. These tests are being repeated.

In all of the experiments except the three just commented upon there was no visible indication of bacterial disintegration

TABLE II

Control experiments. Enumeration of bacteria in inoculated medium containing heated (coagulated) egg albumin and 1 per cent of peptone

ORGANISMS	IMMEDIATELY	24 HOURS	48 HOURS	72 HOURS	4-7 DAYS	2 WEEKS	3 WEEKS	4 WEEKS AFTER INOCULATION
Prot. vulgaris I	11	17,300			XX			
Prot. vulgaris II....	1,472	XX		XX				
Prot. vulgaris III...	46	21,000+						
Prot. vulgaris IV...	1				XX			
B. subtilis.....	2		1,900			XX		XX
Staph. aureus I.....	680	XX		XX				
Staph. aureus II....	94	13,000						
Staph. aureus III...	1				XX			
B. coli.....	1				XX			
B. typhi.....	256		XX					

NOTE: XX indicates too many plate colonies to count.

In all of the experiments recorded in tables I and II the test medium contained the following inorganic salts: Sodium chloride 0.5 per cent, sodium sulphate 0.2 per cent, calcium chloride 0.1 per cent, and acid potassium phosphate 0.1 per cent.

of the egg albumin. In fact, the liquid portion of the medium remained clear and colorless, and the medium could not be distinguished from the uninoculated tubes, either by its appearance to the naked eye or by the odor. Control tubes containing the same ingredients plus 1 per cent peptone rapidly underwent marked change. The protein became involved and, in the tubes containing gelatin-liquefying organisms, was gradually digested. In every instance the liquid part of the medium soon became turbid, and frequently more or less colored (see Table II).

Tests were also conducted with anaerobes of the type of *B. putrificus* and *B. oedematis*, which are characterized by their strong proteolytic and putrefactive properties. No quantitative bacterial determinations were attempted with these anaerobes, however, and the observations were confined to a study of the physical characters of the medium. No change whatever could be noted in the medium; the clear liquid and the coagulated albumin remaining apparently unaffected even after three to four weeks of incubation. Inoculation of egg-meat medium from these tubes with the aid of a platinum loop clearly demonstrated the presence of putrefactive anaerobes by the rapid and characteristic decomposition which took place in the standard egg-meat medium.

II. THE BEHAVIOR OF BACTERIA TOWARD PROTEOSES AND PEPTONES

In text books and other bacteriological publications the assumption is made that proteoses and peptones are readily attacked by all known bacteria which are easily cultivated on artificial media. So firmly has "peptone" established itself as an important ingredient of the common and standard bacteriological laboratory media that its value as the source of nitrogen supply in the cell metabolism of bacteria is taken as a matter of course. It is true that meat extract which is practically protein-free is also looked upon as practically indispensable, but not because it furnishes food as such to the organisms. By many at least it is regarded as a stimulator of cell metabolism, due to the various extractives present.

It is one of the objects of this paper to show that proteoses and peptones follow essentially the same law of resistance to direct bacterial action as do the native proteins, egg albumin, serum albumin and edestin. While the scope of the investigation has as yet been somewhat limited, sufficient data appear to us to have been acquired to warrant their publication at this time.

It is a well-known fact that the proteoses and peptones resulting from the action of proteolytic agents like pepsin and trypsin upon native proteins, and indeed all proteoses and peptones, have

thus far resisted all attempts to isolate or purify them. Hence, it has been impossible to employ all the methods of investigation in a study of their bacteriological-chemical relations which are applicable in connection with certain albumins, as for instance egg albumin. Peptones are now regarded as amino acid combinations of varying complexities, rather than proteins. Witte's peptone, which is essentially a mixture of albumoses and peptones, is far from being made up purely of these nitrogen complexes, although it has long been regarded as the standard for bacteriological purposes. The various American brands are undoubtedly even less pure than the Witte product. It does not follow, however, that they are of correspondingly less value as food for bacteria.

In our study of the behavior of various types of bacteria towards proteoses and peptones the Biuret test for proteins has been employed to great advantage. The method which has been advocated and used by Vernon (1904) for the quantitative estimation of peptone has, with slight modifications been employed by us in the present investigation and in the experiments on bacterial autolysis. A brief description of this method is given here.

The tests were made in Nessler tubes. One cubic centimeter of the inoculated culture fluid was added to 20 cc. of a 4 per cent solution of sodium hydroxide and 2 cc. of a centinormal solution of copper sulphate. To the same mixture of alkali and copper sulphate in a second tube a standard solution (0.25 per cent) of Witte's peptone was added until the same degree of color was produced as in the test medium. The quantity of peptone required in matching the colors was taken as a measure of the amount of peptone present in the inoculated and incubated culture fluid. For example, if 1 cc. of standard peptone solution was required the value of the biuret test was recorded as 1.0, since both liquids gave the same color in the same concentration. Test fluids and controls contained the same amount of Witte's peptone at the outset as the standards, namely 0.25 per cent.

Peptone solutions containing from 0.2 to 2.0 per cent of Witte's peptone were at first employed as culture media for the different

organisms, but it was soon found that the amount of peptone present should not exceed 0.25 per cent. When the peptone was used in higher concentration slight reductions in the amount of the proteins could not be detected, or at least could not be determined accurately. In the lower dilutions, however, the various degrees of decomposition were easily observed.

For the autolysis experiments standard peptone solutions were frequently employed for color comparison, while the work on the relation of the growth of different bacteria to proteoses and peptones involved the employment of the standard solution only as a check or control for the inoculated flasks. The results are not given in per cent, but are represented in the tables by 0, X, XX, XXX and XXXX. The first of these symbols, 0, indicates no reduction of the proteoses and peptones, as compared with the controls, X a slight decomposition, XX fair, XXX strong, and XXXX complete reduction of these soluble proteins. Besides the "peptone" the test media often contained other agents, as will be seen in the tables, namely ammonium sulphate, beef extract and glucose. Furthermore, all of the fluids contained 0.5 per cent of sodium chloride.

The results require but little comment. With few exceptions, no disappearance of albumoses and peptones could be noted in flasks which were inoculated with members of the colon-typhoid group of organisms, even after four weeks of incubation. In the flasks showing a reduction of the biuret reaction the apparent loss of the soluble proteins was slight, and may be accounted for by other factors than an actual decomposition by the bacteria with which they were inoculated. In all of these experiments the bacterial growths were fairly luxuriant, particularly when the test medium contained beef extract or ammonium sulphate. Even in those instances in which slight reduction of the soluble proteins was recorded, at least two weeks, and as a rule three weeks or more, were required to show the apparent reduction.

The above experiments are being repeated. Similar tests are also being made with media containing peptone and the ingredients of the Uschinsky medium, with soluble purified casein, or nutrose and with dialyzed proteoses. Thus far results similar

TABLE III

Showing the behavior of certain gelatin-non-liquefying bacteria towards Witte's peptone

ORGANISMS	MEDIA	DECOMPOSITION OF PROTEOSES AND PEPTONES							
		At 37° C.				At 20° C.			
		1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
B. coli	2 per cent peptone	0	0	0		0	0	0	0
	0.5 per cent peptone	0	0	0	0	0	0	0	0
	0.25 per cent peptone	0	0	0	0	0	0	0	0
B. coli (H).	0.25 per cent peptone	0	0	0	0	0	0	0	0
	0.25 per cent peptone, 0.5 per cent beef extract	0	0	X	X		X?	0	
	0.25 per cent peptone, 1.0 per cent glucose	0		0	X0	0		0	0
	0.25 per cent peptone, 0.25 per cent ammonium sulphate		0	0	0		X	X	X
B. coli (U).	0.25 per cent peptone	0	0	X	X0	0	0	0	0
	0.25 per cent peptone, 0.5 per cent beef extract		0	X	?		0	X	?
	0.25 per cent peptone, 1.0 per cent glucose	0		0	0	0		0	0
	0.25 per cent peptone, 0.25 per cent ammonium sulphate		0	0	X0		0	0	X0
B. coli (A).	0.25 per cent peptone	0	0	0	0	0	0	0	0
	0.25 per cent peptone, 0.5 per cent beef extract		0	X			0	X	
	0.25 per cent peptone, 1.0 per cent glucose	0		0	X	0		0	X
	0.25 per cent peptone, 0.25 per cent ammonium sulphate	0	0	0	0		0	X	X
B. typhi (Y. M. S.)	0.25 per cent peptone	0	0	0	0		X	0	0
	0.25 per cent peptone, 0.5 per cent beef extract		0	X	X	0	0	X	X
	0.25 per cent peptone, 1.0 per cent glucose	0	0	0	0	0	X	XXX	XX
	0.25 per cent peptone, 0.25 per cent ammonium sulphate		0	0	0		0	0	0
B paratyphi B.	0.25 per cent peptone	0	0	0	0	0	0	0	0
B. pullorum	0.25 per cent peptone	0	0		X	0	0		X
B. aerogenes	0.25 per cent peptone	0	0		0	0	0		0

to those already recorded have been obtained. These will constitute part of a future publication from this laboratory.

The results given in Table IV are in marked contrast to the preceding. The pronounced and rapid decomposition of the soluble proteins left no doubt as to the ability of gelatin-liquefying bacteria to convert them into products which no longer give the biuret reaction. *Sp. cholerae* and *Staphylococcus aureus* were, however, much less active than the others.

It may be of interest to note the sparing action of glucose on the proteoses and peptones in the flasks which were incubated at 37°C. At room temperature (20°C.) the protein-sparing action did not last beyond the first two weeks.

III. BACTERIAL AUTOLYSIS

The term "autolysis" has been used somewhat indiscriminately by bacteriologists. Whether it is to denote actual decomposition of the intracellular proteins by the action of the bacteria themselves, or of certain enzymes, or whether it is meant to signify merely a liberation of intracellular substances without change in their chemical structure, is often left undetermined. The word has for many years had definite significance, however, in biochemical literature, carrying with it the idea of self-digestion, as the term implies. This can, of course, be its only true meaning.

It has frequently been shown that real bacterial autolysis is a common phenomenon in organisms of the *Bacillus prodigiosus* and *Bacillus pyocyaneus* type which elaborate strong proteolytic enzymes (Rettger, 1904; Levy and Phersdorff, 1902), especially under conditions of food deprivation and relatively high temperatures. It is to be questioned, however, whether the so-called "autolysis" of cultures of *B. coli* and *B. typhi* during long incubation, and the liberation of endotoxin, as claimed by some investigators (Conradi, 1903) is a process of real self-digestion.

The present study of autolysis was carried on with certain well-known proteolytic organisms, namely *B. prodigiosus*, *Proteus vulgaris*, *Ps. fluorescens* (*B. fluorescens liquefaciens*, Flügge), *B. subtilis* and *B. ramosus*, and with *B. typhi* and several different strains of *B. coli* representing the gelatin-non-liquefying class

TABLE IV

Showing the behavior of certain gelatin-liquefying bacteria to Witte's peptone

ORGANISMS	MEDIA	DECOMPOSITION OF PROTEOSES AND PEPTONES							
		At 37° C.				At 20° C.			
		1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
Staphylococcus aureus.	0.5 per cent peptone	0	0	0	0	0	0	0	0
	0.25 per cent peptone	0	X		X	0	X		X
Staph. albus...	0.25 per cent peptone	XXXX	XXXX		XXXX	X	XXX		XXXX
Sp. cholerae...	0.25 per cent peptone	0	0		XX	0	?		XX
B. ramosus....	0.25 per cent peptone	no growth				0	0		XXXX
B. cereus.....	0.25 per cent peptone	XXXX	XXXX		XXXX	X	XXXX		XXXX
Proteus vulgaris (S)...	0.5 per cent peptone	X	Color interference			X	XX	XXX	
	0.25 per cent peptone								
	1.0 per cent glucose	0	0	0	0	0		XXXX	XXX
	0.25 per cent peptone								
	0.25 per cent ammonium sulphate		XXXX	XXXX	XXXX		XXXX	XXXX	XXXX
Proteus vulgaris (F)	0.25 per cent peptone								
	1.0 per cent glucose	0	0	0	0	0	0	XXX	XXX
	0.25 per cent peptone								
	0.25 per cent ammonium sulphate		XXXX	XXXX	XXXX		XXXX	XXXX	XXXX
Proteus mirabilis..	0.25 per cent peptone	XX	Color interference			0	XX	XXX	XXXX
	0.25 per cent peptone								
	1.0 per cent glucose	0	0	0	0	0	0	XXX	?
	0.25 per cent peptone								
	0.25 per cent ammonium sulphate		XXXX	XXXX	XXXX		XXX	XXX	XXXX

B. subtilis...	0.25 per cent peptone	XXXX	XXXX	XXXX	XXXX	0	XXX	XXXX	XXXX
	0.25 per cent peptone 0.5 per cent beef extract		XXXX	XXXX	XXXX		XXX	XXXX	XXXX
	0.25 per cent peptone 1.0 per cent glucose	0	0	0	0	XX	0	XXX	XXXX
	0.25 per cent peptone 0.25 per cent ammonium sulphate		XXXX	XXXX	XXXX		XXX	XXXX	XXXX
B. prodigiosus.....	2.0 per cent peptone	0	XX	XX		0	0	0	0
	0.5 per cent peptone	XX	XXXX	XXXX	XXXX	X	XXX	XXX	XXX
	0.25 per cent peptone 1.0 per cent glucose	0	0	?	0	X	X	XXX	XX
	0.25 per cent peptone 0.25 per cent ammonium sulphate		XXXX	XXXX	XXXX		XXX		XXXX

NOTE. In all of these tables 0 indicates no reduction in the amounts of proteoses and peptones, and XXXX the complete disappearance of these proteins from the media, as indicated by the biuret test.

of bacteria. The experiments fall into two distinct groups; in the first, tests were made as to the ability of bacteria to digest or destroy their own proteins under highly favorable conditions of temperature and environment. Representative gelatin-liquefying and non-liquefying organisms were employed. The second set of experiments had as its object a study of the fate of purified egg albumin, Witte's peptone, and dialyzed proteoses when added in small amounts to autolyzed bacterial suspensions or to suspensions which had the necessary conditions for proteolysis providing the organisms were capable of digesting themselves.

The different organisms were grown on slant agar and, in a few instances, on potato. The surface growths were washed off with distilled water and transferred to sterile bottles. These suspensions which were of the consistency of a thin paste were incubated at 37°C. with 5 per cent toluol. Definite amounts of this material were tested from time to time by the quantitative biuret method, a 0.25 per cent solution of peptone being employed as a standard for color comparison. The exact plan of the experiments and the results are given in the following tables.

TABLE V

Autolysis experiments with gelatin-liquefying bacteria

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH STANDARD PEPTONE SOLUTION (0.25 PER CENT)									STANDARD PEPTONE (0.25 PER CENT)
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	15th day	
B. subtilis.....	0.05						0.0			
	0.25									
	0.07		Faint			0.0				
										1.0
B. prodigiosus...	5.0	4.5		1.0		0.05				
	2.0		1.0		Faint		0.0			
Prot. vulgaris ...	2.0		1.5		1.0		0.5		Faint	
	1.0		Faint				0.0			
	1.0		Faint				0.5			
B. ramosus.....	0.15	0.10	0.03							

NOTE: The above figures are based on the relative strengths of color obtained in the tests, each being compared with the degree of color given by 1.0 cc. of the standard (0.25 per cent) solution of Witte's peptone which is taken as 1.0. In all of the tests 1.0 cc. of the autolysis material was employed.

TABLE VI

Autolysis experiments with different strains of Bacillus coli

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH STANDARD PEPTONE SOLUTION (0.25 PER CENT)														STANDARD PEPTONE
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	9th day	12th day	14th day	18th day	20th day	30th day	50th day	
B. coli I	2.0	2.0	2.0		?		1.0*								
B. coli II	1.0		1.0		1.0			1.0	1.0						
B. coli III	1.0		1.0			1.0	1.0	1.0	0.75†						1.0
B. coli IV	2.5			2.5								2.5†			
B. coli V	1.0			1.0								1.0			
B. coli VI	1.0	1.0	1.0									1.0			
B. coli VII	1.0								1.0						
B. coli VIII	0.1								0.1		0.1		0.1	0.1	

*Contamination with Subtilis-like organism.

† Developed a condition which rendered the test fluid cloudy, and hence made quantitative study impossible.

TABLE VII

Combined autolysis experiments with gelatin-liquefying and non-liquefying bacteria

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH STANDARD PEPTONE SOLUTION (0.25 PER CENT)															STANDARD PEPTONE
	1st day	2nd day	3rd day	4th day	6th day	7th day	8th day	10th day	25th day	30th day	40th day	75th day	110th day	120th day		
B. subtilis I	1.0		0.0													
B. subtilis II	0.2		0.0													1.0
B. subtilis	0.2					0.0										
Ps. fluorescens	0.5			0.3				0.1		0.0						
B. coli (H)	0.4	0.4		0.4			0.4		0.4	0.4	0.4	0.4	0.4	0.4		
B. immobile (fluorescens non-liquefaciens)	0.6			0.6				0.6		0.6						
B. typhi (Ho)	0.8		0.8		0.8			0.8*	0.0*							
M. luteus (cereus flavus)	0.5		0.5			0.5			0.5							

* At the end of ten days of incubation of the autolysis bottle (B. typhi) 2 cc. of completely autolyzed B. subtilis material was added. After eight days the contents of the bottle failed to give a biuret reaction.

In the following experiments 1 cubic centimeter of autolyzed material or of bacterial suspensions which had been incubated long enough to allow of self-digestion, was added to 5 cc. of purified egg albumin, to Witte's peptone and to purified proteose. Biuret tests were made after definite periods of incubation at 37°C. In each experiment the original biuret reaction is the same for all of the flasks including the control.

TABLE VIII

Proteolytic action of autolysis material on purified egg albumin. 1.0 cc. of suspension added to 5.0 cc. of the albumin solution

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH EGG ALBUMIN CONTROL				
	1st day	3rd day	5th day	12th day	20th day
B. prodigiosus	1.0		0.5	Faint	0.0
B. coli I	1.0	1.0	1.0	1.0	1.0
Control	1.0	1.0	1.0	1.0	1.0

TABLE IX

Action of autolysis material (1.0 cc.) on solution of purified proteoses (5 cc.)

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISON WITH PROTEOSE SOLUTION CONTROL				
	1st day	2nd day	3rd day	6th day	10th day
B. prodigiosus I	1.0		Faint		
B. prodigiosus II	1.0		Faint		0.0
Prot. vulgaris I	1.0	0.5	?	Faint	
Prot. vulgaris II	1.0			0.5	
B. coli I	1.0	1.0	1.0	1.0	1.0
B. coli II	1.0	1.0	1.0	1.0	1.0
B. coli III	1.0	1.0	1.0	1.0	1.0
B. coli IV	1.0	1.0	1.0	1.0	1.0
Control	1.0	1.0	1.0	1.0	1.0

TABLE X

Action of autolysis material (1.0 cc.) on solution of Witte's peptone (5 cc. of a 5 per cent solution). Gelatin liquefiers

ORGANISMS	BIURET REACTIONS AND COLOR COMPARISONS WITH PEPTONE CONTROL					
	1st day	2nd day	4th day	6th day	12th day	20th day
B. prodigiosus.....	5.0	4.0	2.0			
B. prodigiosus II....	5.0	4.0	2.5	1.0	0.5	Faint
Prot. vulgaris.....	5.0	4.0	3.0	2.0	1.5	1.0
Control.....	5.0		5.0		5.0	5.0

NOTE: The controls were prepared by adding 1 cc. of distilled water to 4 cc. of the given peptone solutions.

TABLE XI

Action of autolysis material (1 cc.) on solution of Witte's peptone (5 cc. of a 0.25 per cent solution). B. subtilis and B. coli comparison

ORGANISMS	BIURET REACTION AND COLOR COMPARISONS WITH PEPTONE CONTROL				
	1st day	2nd day	4th day	8th day	12th day
B. subtilis I.....	0.25	0.13	0.08	0.0	
B. subtilis II.....	0.25	0.20		0.12	
B. coli I.....	0.25	0.25		0.25	0.25
Control.....	0.25	0.25		0.25	0.25

NOTE: This experiment was repeated with larger amounts of peptone. The results were practically the same as in this table.

The above tables show clearly the ability of bacteria to digest themselves. This property appears to be confined, however, to organisms which are known to elaborate a proteolytic enzyme—the gelatin-liquefying group. Not only do the organisms of this group rapidly destroy their own protein under favorable conditions of autolysis, but they readily attack and decompose egg albumin, peptones and partially purified proteoses when the autolyzing materials are brought in contact with these foreign proteins.

On the other hand, the gelatin-non-liquefying organisms employed in these experiments were unable to effect any change in the protein content of the respective suspensions, at least in so far as may be judged by the biuret tests. Furthermore, other

proteins, when added to the bacterial suspensions after periods of preliminary incubation, remained unaffected. In every instance where the test was satisfactorily carried out the quality and degree of color obtained in the biuret test remained unchanged, as is readily seen by comparisons with the controls or with the standard peptone solution.

Autolysis of the bacterial cells was always accompanied by a change in the staining properties of the individual organisms. In many cases, as for example in the complete autolysis of *B. subtilis* material, the bacilli took on only a faint color; and the presence of numerous fine granules presented a picture far from the normal. A difference in staining properties was also occasionally observed in the organisms of the *B. coli* type, but this was never marked, and was not due to actual destruction of the cell protein, as was shown always by the biuret test. The change was due to some process other than autolysis, as for instance "washing" or "laking" of the bacterial cells.

GENERAL DISCUSSION AND CONCLUSIONS

The results of the present investigation strongly indicate that bacteria are unable to attack and bring about the decomposition of proteins without the aid of enzymes or other proteolytic agents. This applies not only to the more complex proteins like egg albumin, but in all probability to albumoses and peptones as well. Coagulated albumin shows the same resistance to the direct action of bacteria of both the gelatin-liquefying and non-liquefying types as the unheated and unchanged native proteins.

By means of the quantitative biuret test of Vernon the disappearance of proteoses and peptones from solutions serving as test or culture media may be readily demonstrated. This method has been of much value to us in the present investigation. It is being employed for the determination of other proteins also, as for instance casein in the form of nutrose.

Even during prolonged incubation of flasks containing the necessary inorganic salts for bacterial metabolism, together with proteoses or Witte's peptone, little if any loss of these soluble

proteins could be observed if the flasks had been inoculated with members of the colon-typhoid group or with other gelatin-non-liquefying bacteria. On the other hand, organisms which are known to elaborate proteolytic enzymes, as for example *B. prodigiosus* and *B. subtilis*, rapidly brought about destruction of the proteins. Test media containing purified coagulated egg albumin or dialyzed proteoses as the only possible source of available nitrogen were, with few exceptions, not attacked, however, even by the gelatin-liquefyers, if the inoculations were made with but comparatively few organisms and from a culture less than twenty-four hours old.

The slight reduction in the amount of "peptone" which was observed in a few instances may have been due to agents other than enzymes or bacterial cells, as for instance acids and ammonia. It is significant that such reductions did not become apparent until at least two to three weeks after the time of inoculation. These slight losses in the soluble proteins, if they were losses, usually occurred in flasks containing luxurious growths, and may possibly be due to adsorption by the bacteria and other suspended matter and by the walls of the flasks which were more or less coated. The possibility of the occurrence of small amounts of a proteolytic enzyme having the properties of erepsin (Cohnheim, 1901, Vernon, 1904) cannot be ignored. However, if such an enzyme is produced by organisms of the *B. coli* and *B. typhi* type it is of little importance, as no indications of any proteolytic action whatever were apparent during the first two weeks, and since only very minute quantities can be produced even under the most favorable cultural conditions.

The statement that purified albumin and dialyzed proteoses were not attacked even by gelatin-liquefying bacteria if the test fluids were inoculated with few organisms taken from very young cultures may appear at first paradoxical. The results, which are in harmony with those of Bainbridge and the earlier investigations in this laboratory on purified albumins, readily admit of an explanation. When the test medium contains no other possible source of nitrogen for cell metabolism besides the purified protein it is not attacked by any bacteria unless a sufficient

amount of the inoculating material is introduced to carry with it the necessary enzyme to bring about cleavage of the protein. In a medium containing nitrogen which is directly available, bacterial multiplication will take place, though the number of bacteria introduced is small. If such a medium also contains protein, and if the organism is one which under favorable conditions elaborates a proteolytic enzyme, the protein undergoes cleavage as the result of the enzyme action. These points have been demonstrated repeatedly.

What are some of the important sources of nitrogen available for bacterial metabolism without the aid of an enzyme? Our attention will naturally be directed to amino acids which in animal physiology are now known to play such an important part in nutrition. Witte's peptone contains amino acids which may be demonstrated readily by any of the well-known tests, particularly the Sörensen method (Sörensen, 1908). The amount of amino acids present in the American brands of peptone is considerably greater than in the Witte product. This undoubtedly explains why we have consistently obtained more luxuriant, though not necessarily more characteristic, bacterial growths in media which contained the American products than in the standard Witte.

It appears at this time indeed probable that so-called "peptone media" largely owe their value as culture media to the amino acids and perhaps other nitrogenous substances which readily give up their nitrogen as the result of direct bacterial action, and unless bacteria are present which elaborate proteolytic enzymes, little if any of the proteoses and peptones in the medium is utilized. Indeed it may be necessary for us to go even further than this, and to adopt the view that the bacterial cell can not utilize any protein until after it has been broken up by some other agent and the nitrogenous portion converted into simple form. If this view should obtain it will be necessary for us to alter materially our conception of the value of peptone, nutrose, and other soluble as well as insoluble proteins as culture media, especially in so far as the group of gelatin-non-liquefying bacteria is concerned.

Such a view as is tentatively presented here is certainly in harmony with the results of Loewi (1902) Abderhalden and others who in elaborate investigations have shown that animals like the dog may be maintained in nitrogenous equilibrium for long periods of time when fed on a diet in which all protein material had been replaced by the products of prolonged digestion of proteins. These experiments imply that the cleavage products of the proteins are resynthesized in the animal body. According to Abderhalden no cells can directly assimilate and utilize foreign food material. Complex nitrogenous food material must be prepared for the cell through enzyme action. This breaking down and the reconstruction of food are just as necessary as it is to reduce a church to the very bricks which constitute it before it can be converted into a school-house. This may perhaps apply to the bacterial cell as well as in the field of animal cell nutrition.

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STUDIES ON SOIL PROTOZOA AND THEIR RELATION TO THE BACTERIAL FLORA. I[1]

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I. INTRODUCTION

The occurrence of protozoa in soil

The knowledge that protozoa occur in soil dates back nearly as far as does the science of microbiology, but it is only recently that specific studies have been directed at the micro-fauna of the soil. Müller (1887) reported studies concerning some soil protozoa which he thought played a part in the destruction of organic tissue, and thus were to be considered as important agents in the formation of humus. Celli and Fiocca (1894) studied the protozoa of the soil and described several forms of amoebae obtained from this source. Beijerinck (1896) described an amoeba which occurred in his cultures of nitrifying bacteria, and later (1901) called attention to a variety of amoebae, monads and infusoria which appeared in cultures with Azotobacter. Frosch (1909) isolated a number of saprophytic amoebae from garden soil similar to those found in the intestinal tracts of animals. Tsujitani (1908), likewise described some amoebae which occur in soil. Hiltner (1907) noted many types of protozoa which appeared in cultures made from soil, and stated that these organisms certainly do not play an unimportant rôle. He noted the presence of various ciliates, flagellates, and amoebae, some of which he said were often present in unusually large numbers. Störmer (1907) also studied the protozoan fauna, and demonstrated that the soil contains a considerable number of these organisms, especially amoebae.

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Within the past few years more attention has been directed toward the soil protozoa with the result that they have been demonstrated to be of general occurrence in the soils of those parts of the world which have been studied. In England, Russell and Hutchinson (1909) (1913), Russell and Golding (1912), Goodey (1911), Martin (1912), and Martin and Lewin (1914) have noted the constant presence of protozoa in soil. Similar observations have been made by Wolff (1909) (1912), Francé (1911), Killer (1913), Emmerrich, Leiningen and Loew (1912), and Cunningham and Löhnis (1914) in Germany; by Lodge and Smith (1912), Gainey (1912), Rahn (1913) and Sherman (1914) in the United States; by Cauda and Sangiori (1914) in Italy, Peck (1910) in Hawaii, and Greig-Smith (1912) in Australia; while Loew (1911) (1913) has observed them in the Alps, in Porto Rico, the Island of Borkum, and in Japan. Important contributions to our knowledge of the types of protozoa which occur in soil have been made by Wolff (1909), Francé (1911), Goodey (1911) (1914), Martin (1912), and Martin and Lewin (1914).

The relation of protozoa to bacteria

It is well known that bacteria constitute the chief food for many types of protozoa. Many of the ciliates in particular are especially destructive to bacteria, although this property is possessed by the other classes as well. Indeed, Calkins (1901) has said that probably all protozoa ingest bacteria with the exception of the parasitic forms and those which live on other protozoa. This view, however, has been modified considerably in recent years and it is now known, as is stated by Minchin (1912), that a number of protozoa are saprozoic in nature and obtain their food by absorption. A considerable portion of the non-parasitic flagellates belong to this class.

In many places in nature bacterial development is limited by the action of predatory protozoa. Huntemüller (1905) and Korshun (1907) have proven that the micro-fauna plays an important part in the purification of water. The possibility that protozoa are inimical to the soil bacteria has only very

recently received serious consideration, probably because of the lack of evidence that these organisms exist in an active, free-living condition in the soil.

The phagocytic theory of soil fertility

Interest in the soil protozoa was given a great stimulus in 1909 when Russell and Hutchinson (1909) of the Rothamsted Experimental Station announced their theory which involves the protozoa as a factor detrimental to the soil bacteria, and, therefore, to soil fertility. This theory, commonly known as the phagocytic theory of soil fertility, was proposed in an effort to explain the phenomena associated with the partial sterilization of soil with heat or with volatile antiseptics. The increased yields of crops obtained after partial sterilization is explained, by the sponsers of this theory, on the view that the soil protozoa prey upon the bacteria and thus act as a limiting factor on the microflora of the soil. The process of partial sterilization is thought to destroy the protozoa while the bacteria are greatly reduced, but not exterminated. When the protozoa are suppressed, the bacteria which remain are allowed to multiply unhindered and so attain numbers greatly in excess of those found in normal soils. A greater number of bacteria results in the elaboration of a greater amount of plant food, hence larger crops are produced.

No direct proof has been produced in support of this theory, but Russell and Hutchinson and their associates (1909), (1912), (1913) have presented much evidence of an indirect nature which indicates strongly that some biological factor, detrimental to bacteria, does exist in the soil.

Other views on the partial sterilization of soil

In opposition to the protozoan theory of Russell and Hutchinson, several other explanations have been advanced to account for the beneficial effects of volatile antiseptics upon the higher and lower forms of plant life in the soil. Koch (1899) claims that the antiseptic acts as a stimulant directly upon the bac-

terial flora of the soil, and likewise upon the higher plants. In support of this stimulation theory some very convincing data have been furnished by Koch (1899) (1911), Nobbe and Richter (1904), Egorov (1908), Fred (1911), Gainey (1912) and others.

The selective theory of Hiltner and Störmer (1908) holds that volatile antiseptics exert a selective action on the bacterial flora of the soil. It is thought that the soil is so changed that the subsequent development of the beneficial types of bacteria is enhanced, while the harmful forms are suppressed. These investigators believed the increased crop yields obtained to be due to the increase in the amount of plant food elaborated by the beneficial bacteria.

Bolley (1910) (1913 a) 1913 b) claims that the improvement of soil by partial sterilization is in many cases due to the destruction of certain parasite fungi which attack the plants and thus hinder their growth and development. Another function performed by volatile antiseptics according to Grieg-Smith (1911) is the liberation of plant and bacterial food through the solution of the "agricere" or soil wax.

Other points have been noted in the works of various investigators which partially account for the action of certain antiseptics in soil exclusive of their effect upon the protozoan fauna. Buddin (1914) has shown that the treatment of soil with sulphur dioxide increases the number of bacteria very appreciably without exterminating the protozoa, while certain other compounds such as pyridine cause an increase in the number of bacteria due to the fact that their decomposition products furnish an excellent source of food for the soil micro-organisms. Hutchinson and MacLennan (1914) have shown that the partial sterilization of soil with caustic lime leads to a chemical breaking down of some of the organic matter of the soil and thus stimulates the subsequent activities of the bacteria. Fred (1915) in his work on the action of carbon bisulphide in soil has demonstrated that all of this compound does not evaporate when added to the soil, but that some of it remains and is changed to sulphates.

Some workers believe that the value of partial sterilization by heat is due to the destruction of soil toxins which limit the

activities of the micro-organisms and plants. Whether toxins occur in soil is a disputed question, but Whitney and Cameron (1904) (1910), Schreiner and Reed (1907 a) (1907 b), and Pickering (1910 b) have demonstrated quite satisfactorily that plant toxins do exist; while Bottomley (1911) and Greig-Smith (1911) have submitted data which point to the existence of bacteriotoxins in the soil.

Others explain the beneficial action of heat by the changes it produces in the soil compounds. Frank (1888), Pickering (1910 a), Lyon and Bizzell (1910), Stone (1912) and others have demonstrated an increase in the amount of soluble plant and bacterial food in partially sterilized soil. Pfeffer and Franke (1896) and Krüger and Schneidewind (1899) have shown an increased assimulation by plants in heated soils. Pickering (1910 b), Wilson (1914) and others have further proven that heat may also produce a toxic compound in the soil, the toxicity increasing with an increase in the temperature used, so that soils heated to very high temperatures have a detrimental effect upon plant growth.

The effect of protozoa on the bacterial flora of the soil

There has been only a limited amount of work done upon the action of the protozoa in soil aside from the indirect evidence which has been acquired in the study of partial sterilization. Russell and Hutchinson (1913) have failed in their attempts to reduce the number of bacteria in partially sterilized soil by the addition of mass cultures of protozoa; Lipman, Blair, Owen and McLean (1910) in their work on ammonification in soil were unable to detect any influence upon this process due to the protozoa; while Grieg-Smith (1912) obtained entirely negative results in his efforts to show that protozoa are detrimental to the bacterial flora of the soil. Cunningham (1914), on the other hand, claims to have demonstrated that protozoa do limit the number of bacteria in soil.

Outline of work undertaken

Preliminary to any work on the part played by the soil protozoa, two essential points should be established: (1) whether protozoa occur in soil in numbers sufficient to be a factor in soil fertility; and (2) whether protozoa lead a trophic life in the soil. Unless these two points can be settled in the affirmative, it would appear that discussions concerning the rôle played by the micro-fauna of the soil must be considered more or less futile.. The first part of this work was therefore directed toward getting more definite information as to the number of protozoa in soil and the nature of their existence therein.

A study was made of the effect of protozoa upon the bacterial flora of the soil by the isolation of animal-pure cultures of some representative soil protozoa and inoculation into protozoa-free cultures of soil bacteria, in solutions and in soil, and also by the comparison of the activities of bacteria in sterilized soil reinoculated with normal soil and with "protozoa-free soil."

The last part of the work was devoted to a study of the action of volatile antiseptics in soil in an effort to throw some light on the part played by the protozoa.

II. THE NUMBER OF PROTOZOA IN SOIL

Present status

Although the soil protozoa have attracted considerable attention in recent years, few data are at hand showing the number of such organisms actually found in normal soils. Störmer (1907) showed that fertile soils sometimes contain several thousand amoebae per gram as determined by the agar plate method of enumeration. Hiltner (1907) reported the finding of large numbers of protozoa in soil, and said that flagellates and amoebae had been found in numbers reaching millions per gram. He did not report the specific data upon which this statement was based, nor did he give the details of the method by which the numbers of protozoa were determined. Lodge and Smith (1912), on the

other hand, investigated field soils of Massachusetts and claimed that the number of protozoa present would have to be increased many fold in order to be considered a factor in the limitation of bacterial numbers. Gainey (1912) studied the protozoa in Missouri soils and likewise concluded that the number was not sufficient to be a factor in soil fertility. Rahn (1913) by use of the dilution method determined the number of protozoa in Michigan soils. From the limited data submitted by him it would appear that the soil contains about one thousand protozoa per gram. Killer (1913) tested various methods for the determination of the soil protozoa but concluded that all of the methods now known are of limited and doubtful value. Recently Cunningham (1914) has reported studies of German soils which he has demonstrated to contain quite large numbers of protozoa.

Methods

The dilution method, which has been employed to some extent for estimating the number of protozoa in soil, has been used in this work. It is obviously impossible to devise one medium which will favor equally the development of all of the various forms of unicellular animal organisms found in the soil. In the preliminary work undertaken, various media were used with the idea of finding which forms of protozoa are most abundant in soil, and which media are best adapted to those particular forms.

From observations on several media it was early observed that the flagellates make up the greater portion of the protozoan population of the soils which were studied. The survey of media has not been very extensive, but a dilute soil extract has given the most satisfactory results for determining the number of flagellates. This medium is also well suited for the growth of ciliates and amoebae. The use of soil extract seems appropriate as it would appear that the organisms favored by this medium would be the ones most likely to be leading a trophic life in the soil. One per cent hay extract, which has been used by most investigators in the study of the soil protozoa, has not given as

satisfactory results as has soil extract, in the comparison made at this laboratory.

Soil extract prepared by boiling one part of soil in three parts of distilled water, filtering clear, and adding a small excess of $CaCO_3$, has been used. This has been modified by using only one part of soil in nine parts of water and one part of a one per cent hay extract, plus $CaCO_3$. The presence of this small amount of hay extract does not appear to exert any inhibitory effect upon the flagellates, while the ciliates seem to be benefited. In sampling soil not less than ten grams have been taken, and duplicate dilutions have always been made. The cultures have been incubated at 20°–25°C. and examined every few days for a period of about ten days.

Results

The data obtained on twelve Wisconsin soils, representing various types under different treatments, are tabulated in Table I.

TABLE I

Approximate number of protozoa in various Wisconsin soils

NO.	TYPES OF SOIL	TREATMENT OF SOIL	DILUTIONS			
			1/1,000 gram		1/10,000 gram	
			A	B	A	B
1	Clay loam	Apple orchard	+	+	+	+
2	Clay loam	Blackberry patch	+	+	−	+
3	Rich loam	Garden	+	+	+	+
4	Sandy loam	Tobacco field	+	+	+	−
5	Peat soil	Uncultivated	+†	+	+	−
6	Loam soil	Pasture	+	+	−	−
7	Clay loam	Vineyard	+*	+*	−	+
8	Loam soil	Alfalfa field	+	+	−	+
9	Clay loam	Not planted	+*	+*	+	−
10	Sandy loam	Clover	+	+	−	−
11	Sandy loam	Castor bean bed	+	+	−	+
12	Sandy loam	Corn field	+	+	+	+

A and B represent the duplicate samples of each soil.
+ = presence of protozoa.
− = no protozoa present.
* Colpoda found in addition to flagellates.
† Amoebae found in addition to flagellates.

That the high protozoan content noted above is not peculiar to Wisconsin soils is shown by the data obtained from three Virginia soils and one Tennessee soil given in Table II. These samples were from representative soils in a good state of cultivation.

The figures of the foregoing tables show that, in the sixteen soils recorded, in every soil protozoa were found in both samples of the 1/1,000 of a gram dilution, while of the total of 32 dilutions representing 1/10,000 of a gram each, 18 revealed the presence of protozoa. When we consider that, (1) it is extremely improbable that every individual protozoan grows when taken

TABLE II

The approximate number of protozoa in Virginia and Tennessee soils

NO.	SOURCE	TYPE OF SOIL	TREATMENT OF SOIL	DILUTIONS			
				1/1,000 gram		1/10,000 gram	
				A	B	A	B
1	Virginia	Clay	Garden	+	+	+	+
2	Virginia	Clay loam	Wheat field	+	+	−	+
3	Virginia	Loam	Grass field	+	+*	+	+
4	Tennessee	Compact red clay	Corn field	+	+	−	−

A and B represent the duplicate samples of each soil.
+ = presence of protozoa.
− = no protozoa present.
* *Balantiophorus elongatus* found in addition to flagellates.

from the soil and introduced into a liquid medium; (2) the liquid medium used is probably not adapted for the growth of all protozoan inhabitants of the soil; and (3) some of the samples representing only 1/10,000 of a gram contained three distinct types of animal organisms, the statement that the average fertile soil contains about 10,000 protozoa per gram seems conservative.[2]

In cultures inoculated with 1/1,000 of a gram of soil, a variety of flagellates are usually to be observed. As seen from the above

[2] It should be borne in mind that these data are not intended to give an accurate estimate of the numbers of protozoa in soil, but only to show whether they occur in numbers sufficient to be regarded as a possible factor in soil fertility.

data, the ciliates do not ordinarily occur in numbers approximating 1,000 per gram. *Colpoda cucullus* which has been widely noted as an inhabitant of soil, appears to be the most generally distributed ciliate. This organism may be very often, but not constantly, found in 1/100 of a gram of soil. In one Virginia soil (Table II), *Balantiophorus elongatus* was found in the 1/1,000 of a gram dilution. With the methods employed, it would not be safe to draw any conclusions regarding the amoebae other than that they do not occur in numbers nearly as great as do the flagellates in ordinary soils. Although the culture media and period of observation used are not adequate for work with the amoebae, it is worthy of note that in the peat soil, containing a high humus and water content (in which we should expect to find numerous amoebae) the method was of sufficient service to show their presence in 1/1,000 of a gram in one of the two samples taken. In spite of the limitations of the methods used, amoebae would probably have been revealed had they been of general occurrence in large numbers. Using the same medium and inoculating with a large amount of soil, amoebae have been observed as early as the third day.

In the dilutions of 1/10,000 of a gram, with one exception, only four general types of protozoa have as yet been noted: *Monas sp. Dimorpha radiata* (?) and two other flagellates which have not been identified.

III. THE GROWTH OF PROTOZOA IN SOIL

Previous work

In order to correlate the protozoa with any of the vital functions of the soil, it is first necessary to demonstrate that they are active in soils of normal moisture content. Goodey (1911) made a very careful study of the ciliates of the soil and concluded that these organisms exist in soils of normal moisture content only in the encysted condition. When free water was to be found on the soil active protozoa could be demonstrated, but no active ciliates could be detected in ordinary soils. Russell and Golding (1912) working with water-logged, "sewage sick" soil, demon-

strated the presence of active protozoa. Under these conditions, as was pointed out by Goodey, many protozoa may become active. When we consider the minute size of these organisms it would appear obvious that they may become active whenever the soil is in a saturated condition. Martin (1913) by means of a special method which he has developed has been able to prove definitely that soil contains active protozoa. Cunningham (1914) has collected data which indicate that some of the protozoa exist in soil in the active state.

Experimental

In order to see if any of the soil protozoa are active, the following experiment was performed. Four pots each containing two kilograms of sterilized soil with an optimum moisture content of about 17 per cent were inoculated with two grams each of a normal soil, which showed 10,000 protozoa per gram by the dilution method. These pots were then brought up to varying moisture contents by the addition of sterile water, as follows:

	per cent H_2O
Pot No. 1	11
Pot No. 2	12
Pot No. 3	15
Pot No. 4	22

Since one gram of normal soil, with a protozoal count of 10,000 per gram, was used to inoculate 1,000 grams of sterile soil, the resulting mixture should contain, approximately, only 10 of these organisms per gram. After fifteen days incubation at 20 to 25°C., these pots were sampled, and in every case flagellates were found in the 1/10,000 dilutions. This could not be explained except on the assumption that these animal organisms had undergone rapid multiplication. The forms found in greatest numbers in these pots were the same forms which were noted as the predominating types in normal soils. *Monas sp.* did not occur in the 1/10,000 dilution cultures from the soils containing only 11 and 12 per cent moisture. In the case of pot 4, with a moisture content of 22 per cent, *Colpoda cucullus* was found in the

1/100 dilution, showing that this organism was probably active in the soil containing this high amount of water.

To obtain more definite data on this point, with known organisms, an experiment was performed in which pots of sterilized soil were inoculated with animal-pure cultures of three of the types of flagellates mentioned in the first part of this paper. The pots were inoculated with 0.1 gm. each of soil cultures of the respective organisms per 1,000 grams of sterile soil. The water content was then held at 15 per cent (a little below normal for a soil of this type). Determinations for protozoa were made immediately after inoculation, and then at intervals for fifteen days. The results obtained are given in Table III.

TABLE III

The multiplication of three types of protozoa in soil. (H_2O *content 15 per cent.*)

ORGANISM	NUMBER PER GRAM				
	Start	6 days	9 days	12 days	15 days
Monas (sp)	1	10	10	1,000	10,000
D. radiata	1	100	100	1,000	100,000
Flagellate A	10	100	1,000	10,000	100,000

In similar tests using *Colpoda cucullus, Balantiophorus elongatus* and *Oxytricha sp.* no multiplication could be detected during a period of thirty days. These results appear to substantiate the data of Goodey on the ciliates.

A point which probably deserves mention in this connection, is that when sterilized soil is inoculated with normal soil, the protozoan fauna rises in numbers above that of normal soil, just as does the bacterial flora. In other words, it is probable that the micro-organic balance remains about the same. In Table IV are given the results obtained on five pots of sterilized soil which were inoculated with normal soil.

Higher dilutions have not been tried, but it is not unlikely that under such conditions the number of protozoa may reach 1,000,000 per gram. This fact is not of great significance, but it has a practical application in indicating that the subsequent work on the relation of the soil protozoa to the bacterial flora

may be carried out in a sterilized soil medium without causing an apparent disturbance in the balance between these two classes of organisms.

TABLE IV

Approximate number of protozoa in reinoculated sterile soil

POT NO.	DILUTIONS			
	1/10,000 gram		1/100,000 gram	
	A	B	A	B
1	+	+	+	+
2	+	+	+	−
3	+	+	+	+
4	+	+	−	+
5	+	+	+	+

A and B represent the duplicate samples of each pot.
+ = presence of protozoa.
− no protozoa present.

IV. THE BEHAVIOR OF BACTERIA IN SOILS CONTAINING PROTOZOA AND FREE FROM PROTOZOA

Methods

In an effort to show the influence of the soil protozoa upon the bacterial flora the following method was employed. Pots of soil covered with a layer of non-absorbent cotton between layers of cheese cloth, to prevent reinfection, were sterilized in the autoclave under fifteen pounds steam pressure for one hour. Some of the pots were then inoculated with unsterilized soil in order to introduce all of the biological factors peculiar to normal soils, while the others were inoculated with a special soil containing a varied mixture of soil bacteria but free of protozoa.

This "protozoa-free soil" was made up of a mixed flora obtained from several different soils by the isolation of as many kinds of bacteria as could be obtained. For this purpose several different soils were plated out on beef extract, casein, Heyden and Ashby's agars and all of the colonies which developed transferred to sterile soil. Other portions of soil which had been partially sterilized by heat sufficiently to kill all protozoa

were also added to this soil culture. The flora was made more complex by the addition of dilutions of various soils which represented 1/1,000,000 of a gram, and which contained no protozoa. It would seem that the addition of these dilutions would seed the soil culture with those types of bacteria which predominate in normal soils. The protozoa-free soil so prepared was protected from contamination and its freedom from protozoa was verified at frequent intervals.

In all of the experiments in this part of the work at least one kilogram of soil was used in each pot. For most of the trials larger amounts were used according to the size of the pots. The moisture content of the soils was maintained at about two-thirds of their water holding capacities.

For the determination of the number of bacteria the soils were plated on Heyden agar and the counts made after ten days incubation at laboratory temperature.

The number of bacteria in soils containing protozoa and free of protozoa

Many determinations have been made of the number of bacteria in sterilized soils which were reinoculated with normal soil and with the special protozoa-free soil previously described. Before the results from these tests are discussed, it should be recognized that the method is open to severe criticism. A comparison is made of two soils containing quite different flora. It is not to be expected that the flora of the artificial soil used approaches in complexity the bacterial flora of the normal soil. The number of bacteria found in these soils would probably be different even though neither be influenced by any detrimental factor. It is logical to believe that the greater number of bacteria would be found in the soil containing the more complex flora, since it would seem, the greater the variety of bacteria the greater would be the efficiency of the flora in the utilization and destruction of its own by-products.

If this view is correct, the greater number of bacteria should be found in the soil inoculated with normal soil unless the micro-

flora of this soil is limited by some harmful factor. In all of the tests which have been made *the soils free of protozoa contained greater numbers of bacteria than the corresponding soils which were inoculated with normal soil.* The results obtained in a few representative trials are given in Table V.

TABLE V

The number of bacteria in soils free of protozoa and containing protozoa

Test 1*—Incubation period two months

POT	INOCULUM PROTOZOA FREE		INOCULUM NORMAL SOIL	
	Bacteria per gram	Average	Bacteria per gram	Average
1	390,000,000		206,000,000	
2	290,000,000		110,000,000	
3	274,000,000	294,800,000	232,000,000	175,600,000
4	250,000,000		164,000,000	
5	270,000,000		166,000,000	
Test 2—Incubation period one month				
1	340,000,000	307,500,000	106,000,000	110,000,000
2	275,000,000		114,000,000	
Test 3—Incubation period three months				
1	230,000,000	205,000,000	142,000,000	141,000,000
2	180,000,000		140,000,000	

* Each of these tests represents a distinct experiment and not a re-count on the same soil.

An experiment was also performed to see if the above phenomena would occur on different types of soil. For this purpose a rich muck soil, a clay loam and a poor sandy soil were used. The results obtained (Table VI) showed the characteristic difference in each case.

The results obtained in the foregoing experiments can not be considered as proof that the soil protozoa are inimical to the bacteria because of the differences in the two soils under consideration. However, these data, together with the previous observations that the soil contains an adequate supply of protozoa and that some of these organisms are active, certainly appear to add weight to the theory of Russell and Hutchinson that protozoa serve as a limiting factor upon the bacteria in the soil.

TABLE VI

The number of bacteria in different types of soil containing protozoa and free of protozoa

INOCULUM	NUMBER OF BACTERIA PER GRAM		
	Muck soil	Loam soil	Sandy soil
After fifteen days incubation			
Protozoa-free	1,030,000,000	617,000,000	24,200,000
Normal soil	307,000,000	214,000,000	11,900,000
After sixty days incubation			
Protozoa-free	210,000,000	157,000,000	21,800,000
Normal soil	108,000,000	70,000,000	6,700,000

The effect of the complexity of the bacterial flora upon the apparent number of bacteria in soil

As was previously pointed out the weakness in the tests in which the number of bacteria in soils containing protozoa and free of protozoa are compared lies in the fact that the flora of the two soils are different. Whether the complexity of the bacterial flora affects the apparent number of bacteria, as revealed by the agar plate count, is very important in this connection. An experiment was conducted in order to test this point.

Twelve pots of sterilized soil were divided into four groups of three pots each. The soils in group A were then inoculated with all of the bacteria which developed on six Heyden agar plates from two different soils. Group B was inoculated with A plus a mixture of all the bacteria that developed on beef extract, nutrose, and casein agar plates taken from several different types of soil and from different depths of soil. Group C was inoculated with A and B plus the "protozoa-free soil" used in the previous experiments. The pots in group D were inoculated with normal soil. After incubation periods of fifteen and thirty days at laboratory temperature, samples were taken and bacterial counts made using Heyden agar. The results obtained are given in Table VII.

The results in Table VII show very clearly that the bacteria

in soil, as determined by the plate culture method, are diminished with an increase in the complexity of the flora. The soils used were all free of protozoa with the exception of those in Group D, yet there is a continual decrease in the number of bacteria found in each group as the number of kinds of bacteria is increased. It will be noted that there was a greater difference obtained in the bacterial counts between Groups B and C, neither of which contained protozoa, than there was between C and D, one of which contained protozoa while the other was free of these organisms. It is very probable that this decrease in the number of

TABLE VII

Effect of complexity of flora upon the apparent number of bacteria in soil

NO.	NUMBER PER GRAM			
	Group A	Group B	Group C	Group D
Fifteen days				
1	500,000,000	460,000,000	220,000,000	190,000,000
2	600,000,000	420,000,000	240,000,000	250,000,000
3	580,000,000	460,000,000	270,000,000	270,000,000
Average.....	560,000,000	447,000,000	243,000,000	237,000,000
Thirty days				
1	700,000,000	420,000,000	280,000,000	230,000,000
2	580,000,000	420,000,000	250,000,000	200,000,000
3	700,000,000	400,000,000	250,000,000	210,000,000
Average.....	660,000,000	413,000,000	260,000,000	231,000,000

bacteria, due to an increase in the complexity, is only an apparent one and that the actual number of bacteria is just as great as in the soils containing a less complex flora. This view might be explained on the ground that in the more complex flora there was a larger percentage of bacteria which were not able to grow on agar plates. If in two soils each of which actually contained 300,000,000 bacteria per gram but in one 90 per cent of the organisms were able to develop colonies on agar plates while in the other only 50 per cent had this property, the counts obtained would be 270,000,000 and 150,000,000 respectively. Since growth

on agar was the chief means employed to obtain bacteria free of protozoa, there can be no doubt but that the protozoa-free soil used in these experiments contained a higher percentage of bacteria capable of development on agar plates than did the normal soil.

The relation of the number of kinds of bacteria in soil to the total number is in itself a large problem. Whatever the explanation for the variation in the numbers of bacteria, as determined by the plate culture method, with the complexity of the flora may be, it appears very clear that the differences in the numbers of bacteria in the soils with and without protozoa obtained in the foregoing experiments were due in large part, at least, to the complexity of the bacterial flora itself. This casts doubt upon the belief that protozoa act as a limiting factor, but it is possible that the reduction in bacterial numbers in group D was in part due to these organisms.

The effect of temperature upon the development of bacteria in soils with and without protozoa

If the soil protozoa act as a limiting factor upon the bacteria it should be possible to demonstrate that fact by the subjection of soils with and without protozoa to conditions that would inhibit the growth of the protozoa but not that of bacteria. Russell and Hutchinson explain the fact that the soil contains more bacteria during the winter and early spring months than in the summer on the view that the protozoa are not active at such low temperatures. It seems, therefore, that observations on the development of bacteria in soils which contain protozoa and others free of protozoa when exposed to winter weather should throw some light on the subject.

Four pots of sterilized soil were inoculated, two with bacteria alone and two with ordinary soil. The soils were kept at laboratory temperature for two months after inoculation and then placed out of doors during the months of December, January, February and March. The soils remained frozen throughout the greater part of this period. Bacterial counts were made just

before the pots were put out of doors and at the end of 45 days and 105 days periods outside. The data obtained are presented in Table VIII.

If the soil protozoa have a detrimental effect upon the bacteria we should expect the number of bacteria in the pots inoculated with ordinary soil to rise very markedly, while in the soils free of protozoa there should not be such an increase. There was, apparently, no difference in the behavior of the bacteria in the different soils.

TABLE VIII

Effect of low temperature upon the number of bacteria in soils containing protozoa and free of protozoa

POT	WITHOUT PROTOZOA		WITH PROTOZOA	
	Bacteria per gram	Average	Bacteria per gram	Average
Before being placed out doors				
1	300,000,000	232,000,000	77,000,000	75,500,000
2	165,000,000		74,000,000	
Forty-five days after being placed out doors				
1	250,000,000	260,000,000	130,000,000	95,000,000
2	270,000,000		60,000,000	
One hundred and five days after being placed out doors				
1	280,000,000	308,000,000	100,000,000	110,000,000
2	336,000,000		120,000,000	

Another experiment was performed on the relation of temperature to the bacterial flora in the presence and absence of protozoa. Three pots of sterile soil were inoculated with normal soil while three other pots were inoculated with the protozoa-free soil. One pot of the soil from each lot was then incubated at each of three temperatures, 10°C., 22°C., and 37°C. for a period of 30 days. In this case also there should be a difference in the development of the bacteria in the two soils at the various temperatures if soil is possessed of a detrimental biological factor. The results (Table IX), as in the foregoing experiment, give no evidence of a phagocytic agent.

TABLE IX

Number of bacteria in soils kept at different temperatures

INOCULUM	NUMBER OF BACTERIA PER GRAM		
	10°C.	22°C.	37°C.
Without protozoa	460,000,000	360,000,000	210,000,000
With protozoa	110,000,000	220,000,000	150,000,000

In the soils kept at 37°C. there was the same difference between the numbers of bacteria in the soils containing and free of protozoa as in the soils incubated at room temperature. The greatest difference was found in the soils incubated at 10°C., which fact is opposed to the protozoan theory, unless it be assumed that the protozoa act better at low temperatures. Such an assumption is not in accord with the known facts concerning them.

Effect of moisture content upon bacteria in soils with and without protozoa

It is generally acknowledged that protozoa require a larger amount of moisture than bacteria. An experiment was made with soils of a very low moisture content in order to eliminate the action of the "detrimental factor," if such exists in the soil. Soil with an optimum moisture content of 18 per cent was dried so as to contain only 8 per cent water. This soil was sterilized

TABLE X

Number of bacteria in soils of low moisture content

POT	INOCULUM	NUMBER OF BACTERIA PER GRAM		
		30 days	45 days	60 days
1	Without protozoa	590,000,000	660,000,000	484,000,000
2	Without protozoa	770,000,000	654,000,000	390,000,000
Average	Without protozoa	680,000,000	657,000,000	437,000,000
3	With protozoa	270,000,000	170,000,000	70,000,000
4	With protozoa	470,000,000	150,000,000	120,000,000
Average	With protozoa	370,000,000	160,000,000	95,000 000

and then reinoculated, one set without protozoa and the other set with protozoa. The moisture content was held at 8 per cent and samples were taken for bacterial analysis at the end of 30, 45 and 60 days. The results are given in Table X. These show that the relation between the number of bacteria in the presence and absence of protozoa is the same in soils of low water content as when more moisture is present. This fact argues strongly against the protozoan theory.

The development of bacteria in soils containing protozoa and free of protozoa

As is well known, protozoa do not multiply as rapidly as bacteria. It is also the contention of Russell and Hutchinson that the biological factor, which they believe to exist in soils, requires much more time to develop in soil than is necessary for the bacterial flora. If such a factor exists in the soil there should be a difference in the development of bacteria in soils containing protozoa and soils free of protozoa. We should expect the bacteria to multiply very rapidly in each soil and to attain about the same maximum numbers. At a later period, in soil containing protozoa, the number of bacteria should diminish very appreciably, due to the development of the "detrimental factor," while the number of bacteria in the protozoa-free soil should remain much nearer its maximum. It has been observed on a number of occasions that the difference in the number of bacteria which develop in the soils inoculated with normal soil and with protozoa-free soil is apparent fifteen days after inoculation, and also that the difference in the two flora apparently remains the same from the fifteenth day through the third month. (Tables V, VI, VII and VIII.) The harmful factor, if such exists, must develop within fifteen days after the soil is inoculated.

Two pots of a rich garden soil, two of a field soil (loam) and two of a poor sandy soil were sterilized. One pot of each was then inoculated with normal soil and the other pot from each set inoculated with protozoa-free soil. Bacterial counts were made every day for sixteen days. The data obtained are tabulated in Table XI.

A study of the data fails to give any evidence that the protozoa act as a limiting factor upon the soil bacteria. In general it will be seen that curves representing the numbers of bacteria throughout the period would run nearly parallel in the different soils. The difference in the numbers of bacteria in soils with and without protozoa was apparent from the start, which fact indicates that the phenomena is due to a difference in the bacterial flora and not to the protozoa. There was no evidence that a detrimental factor developed during the latter part of the period.

TABLE XI

The development of bacteria in soils containing protozoa and in soils free of protozoa

DAYS	NUMBER OF BACTERIA IN MILLIONS PER GRAM					
	Garden soil		Field soil		Sandy soil	
	A	B	A	B	A	B
1	150	40	350	250	12	15
2	200	30	315	115	12.8	8
3	400	50	400	250	40	12
4	430	275	800	200	38	12
5	1,400	420	600	400	55	25
6	1,300	620	600	410	65	20
7	1,420	760	780	500	50	27
8	1,650	925	900	450	48	21
9	950	770	910	425	36	28
10	830	430	450	220	28	12
11	620	440	530	330	40	14
12	770	360	640	275	32	12
13	1,050	490	730	215	23	9.8
14	1,030	307	617	214	24.2	11.9
15		230	385	175	31.2	11.2
16	845	257	367	204	19.4	7.8

A = soil without protozoa.
B = soil with protozoa.

Discussion

An examination of the data presented in the foregoing experiments reveals the fact that in all of the tests the soils which contained protozoa gave lower bacterial counts as determined

by the plate culture method. This observation supports the theory of Russell and Hutchinson. It must be remembered, however, that the bacterial flora contained in the soils with and without protozoa were quite different; the flora of the protozoa-free soil was not so complex as that of ordinary soil. It was shown that, *under the conditions of these experiments*, the apparent number of bacteria was affected appreciably by the number of kinds of bacteria present. That the difference in the two flora was an important factor in the disparity between the apparent number of bacteria in the soils with and without protozoa can hardly be doubted; the assumption that it was the only factor, however, would not be justified.

If the bacterial flora in these tests was limited by the protozoa, these organisms must be able to grow actively at low temperatures (0°C.), at high temperatures (37°C.), and in soils of very low moisture content (Tables VIII, IX, and X). It would also have to be acknowledged that the harmful protozoa are capable of multiplying as rapidly in soil as the bacteria (Table XI). Such characteristics would be different from those of any protozoa now known. Admitting these possibilities, it must be concluded that the weight of the evidence is opposed to the view that the soil protozoa are harmful to the bacteria.

An objection that may be raised to this work is that sterilized soil was used as a substratum. It may be claimed that the particular kinds of protozoa which are believed, by some workers, to act unfavorably in the soil are not capable of development in sterilized soil. Such a possibility must be recognized, though there is no reason for this belief, while the evidence we have on this subject (see Part III) is opposed to such a view.

A very essential part of this work is the study of the bacterial processess in soils with and without protozoa. This phase of the work was undertaken by another worker in this laboratory the results of whose work will probably be ready for publication in the near future. It may be stated now, however, that the data obtained are in perfect accord with the foregoing observations.

V. THE EFFECT OF PURE CULTURES OF PROTOZOA UPON THE SOIL BACTERIA

Isolation of protozoa

The term "pure culture" as it is here used in connection with protozoa means only an "animal-pure culture," since the cultures of protozoa were not obtained free from bacteria. The soil amoebae, as has been shown by Beijerinck (1896), (1897), Celli (1896), Tsujitani (1908), Frosch (1909), and others, may be cultivated on solid culture media and so can be isolated in the pure state more readily than the other types of protozoa. With the ciliates and flagellates the problem of obtaining cultures free of bacteria is more difficult. Berliner (1909) and Nägler (1909) have shown that certain of the flagellates and ciliates will grow upon agar plates but this method has not given general satisfaction. Many methods have been tried, some of which have been partially successful, but none have proved very practical.

It was found in this work that animal-pure cultures could be conveniently obtained by the dilution method. As was noted earlier in this paper in high dilutions of soil (1/10,000 gm.) the protozoa present are usually restricted to a few types. By inoculation of a large number of high dilutions of soil (1/10,000 – 1/50,000 gm.) into soil extract a few cultures which contain only one type of protozoa will usually be obtained. In order to isolate types which do not occur in soil in very large numbers it is necessary to utilize an enrichment medium which favors the growth of the desired organism. When the desired type has obtained the ascendency, it may be readily isolated by the dilution method. Hay extract, Ashby's solution, Giltay's solution, dilute peptone broth, and a weak beef extract solution have been used successfully for enrichment cultures.

After the protozoa were obtained in animal-pure cultures it was attempted to simplify the bacterial flora contained in them. This was accomplished by passage through several successive subcultures in Ashby's solution. The types of bacteria capable of development in this medium are somewhat limited.

Methods

The effect of the various protozoa isolated upon soil bacteria was tested in soil extract and then in soil. The soil tests were made by the addition of the protozoan cultures to sterilized soils reinoculated with protozoa-free soil. Tumblers each containing 200 grams of soil were used for these experiments, and the soils were incubated 30 days at laboratory temperature, which ranged from 20° to 25°C. The moisture contents of the soils were held as closely as possible to the optimum for the growth of plants and micro-organisms. (The optimum moisture content is generally considered to be very close to the moisture equivalent of the soil.) Inoculations of the soil and soil extract cultures were made by the addition of one cubic centimeter of an active soil extract culture of the protozoan under investigation.

The experiments reported here include the tests which have been made with six types of protozoa, the ciliates, *Colpoda cucullus*, and *Balantiophorus elongatus*, and the four flagellates which have been previously mentioned (Part II) as the most abundant types in soil:—*Monas sp.*, *Dimorpha radiata* (?) and two others designated for convenience as *flagellates A* and *B*.

Results with Colpoda cucullus

In soil extract, as would be expected, the number of bacteria is greatly reduced in the presence of *Colpoda cucullus*. In soil, on the other hand, no such limiting action could be detected, which further substantiates the view that this organism is not active under ordinary soil conditions.

TABLE XII

Effect of Colpoda cucullus upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	2 days	5 days	8 days
Control	100,000,000	140,000,000	144,000,000
Control	128,000,000	142,000,000	100,000,000
Colpoda	102,000,000	9,400,000	5,000,000
Colpoda	110,000,000	9,500,000	4,000,000

TABLE XIII

Effect of Colpoda cucullus upon the number of bacteria in soil

Test 1

POT	CONTROL	AVERAGE	C. CUCULLUS	AVERAGE
1	470,000,000	490,000,000	405,000,000	452,500,000
2	510,000,000		500,000,000	

Test 2

1	1,640,000,000	1,900,000,000	2,640,000,000	2,570,000,000
2	2,160,000,000		2,500,000,000	

Test 3

1	620,000,000	722,500,000	770,000,000	752,500,000
2	670,000,000		730,000,000	
3	820,000,000		810,000,000	
4	780,000,000		700,000,000	

Results with Balantiophorus elongatus

The results obtained with *Balantiophorus elongatus* are similar to those with *Colpoda cucullus;* in liquid cultures the bacteria are definitely suppressed while in soil no such action is evident. The slight difference noted in Table XV is easily within the limits of experimental error. Only one test of *B. elongatus* in soil is here reported; since this organism is not active in soil (Part III) it is obvious that it could not function as a bacterial limiting factor.

TABLE XIV

Effect of Balantiophorus elongatus upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	3 days	5 days	8 days
Control	114,000,000	141,000,000	122,000,000
B. elongatus	136,000,000	60,000,000	9,000,000

TABLE XV

Effect of Balantiophorus elongatus upon the number of bacteria in soil

POT	CONTROL	NUMBER OF BACTERIA PER GRAM		
		Average	B. elongatus	Average
1	620,000,000	722,500,000	700,000,000	612,500,000
2	670,000,000		580,000,000	
3	820,000,000		520,000,000	
4	780,000,000		650,000,000	

Results with Dimorpha radiata

Dimorpha radiata is an active inhabitant of soil and should therefore have some function therein. From the data obtained that function does not appear to be the destruction of bacteria. Neither in soil extract nor in soil did there appear to be any limiting action upon the bacteria.

TABLE XVI

Effect of Dimorpha radiata upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	2 days	5 days	8 days
Control	100,000,000	140,000,000	144,000,000
Control	128,000,000	142,000,000	100,000,000
D. radiata	146,000,000	120,000,000	100,000,000
D. radiata	190,000,000	90,000,000	116,000,000

TABLE XVII

Effect of Dimorpha radiata upon the number of bacteria in soil

Test 1

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	D. radiata	Average
1	1,360,000,000	1,290,000,000	2,050,000,000	1,830,000,000
2	1,220,000,000		1,610,000,000	

Text 2

1	470,000,000	490,000,000	310,000,000	447,500,000
2	510,000,000		585,000,000	

Results with Monas sp.

In the case of *Monas sp.* very interesting results were obtained. This organism, as has been shown, is active in soil. The findings reveal a very strikingly harmful effect upon bacteria in soil extract while in soil this action did not appear to take place. The reason for this is not clear, but it has been noted within recent years that the behavior of micro-organisms apparently differs, in many cases, in soil and in solution.

TABLE XVIII

Effect of Monas (sp.?) upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	2 days	5 days	8 days
Control	100,000,000	140,000,000	144,000,000
Control	128,000,000	142,000,000	100,000,000
Monas (sp.?)	200,000,000	6,500,000	5,500,000
Monas (sp.?)	46,000,000	6,800,000	6,000,000

TABLE XIX

Effect of Monas (sp.?) upon the number of bacteria in soil

Test 1

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	Monas	Average
1	155,000,000		105,000,000	
2	105,000,000	133,300,000	290,000,000	165,000,000
3	150,000,000		100,000,000	

Test 2

1	440,000,000		380,000,000	
2	360,000,000	420,000,000	240,000,000	293,300,000
3	460,000,000		260,000,000	

Test 3

1	240,000,000		240,000,000	
2	170,000,000	204,000,000	165,000,000	221,000,000
3	160,000,000		220,000,000	
4	245,000,000		260,000,000	

TABLE XX

Effect of seven different strains of Monas (sp.?) upon the numbers of bacteria in soil

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	Monas	Average
1	840,000,000		780,000,000	
2	930,000,000		660,000,000	
3	660,000,000		760,000,000	
4	800,000,000	786,000,000	930,000,000	788,600,000
5	700,000,000		940,000,000	
6			710,000,000	
7			740,000,000	

To verify the results of Table XIX, another test was made in which seven strains of the organism, isolated from as many different soils, were employed. A comparison was then made with five control pots which were free of protozoa. The results from this test (Table XX) agree entirely with the data given in the preceding tables.

Results with Flagellate A

This organism, like *Dimorpha radiata*, does not appear to be antagonistic to the soil bacteria as is shown by the tests both in soil and in soil extract.

TABLE XXI

Effect of Flagellate A upon the number of bacteria in soil extract

	NUMBER OF BACTERIA PER CUBIC CENTIMETER	
	3 days	5 days
Control	57,000,000	31,000,000
Control	77,000,000	35,000,000
Flagellate A	95,000,000	31,000,000
Flagellate A	58,000,000	50,000,000

TABLE XXII

Effect of Flagellate A upon the number of bacteria in soil

Test 1

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	Flagellate A	Average
1	1,530,000,000	1,520,000,000	2,660,000,000	2,445,000,000
2	1,510,000,000		2,230,000,000	

Test 2

1	825,000,000	862,500,000	725,000,000	685,000,000
2	900,000,000		645,000,000	

Test 3

1	136,000,000	129,000,000	136,000,000	141,000,000
2	122,000,000		146,000,000	

Results with Flagellate B

The organism used in this test, designated as *Flagellate B*, is an active inhabitant of the soil, in which it occurs in numbers nearly as great as the three preceding flagellates. This organism does not appear to affect the number of bacteria, as determined by the plate culture method, either in soil nor in soil extract cultures.

TABLE XXIII

Effect of Flagellate B upon the number of bacteria in soil extract

Period of incubation five days

CULTURE	NUMBER OF BACTERIA PER CUBIC CENTIMETER			
	Control	Average	Flagellate B	Average
1	23,000,000	33,000,000	39,000,000	38,000,000
2	43,000,000		37,000,000	

TABLE XXIV

Effect of Flagellate B upon the number of bacteria in soil

POT	NUMBER OF BACTERIA PER GRAM			
	Control	Average	Flagellate B	Average
1	240,000,000	203,800,000	195,000,000	185,000,000
2	170,000,000		165,000,000	
3	160,000,000		210,000,000	
4	245,000,000		170,000,000	

Mixture of Protozoa

It was thought that, although none of the individual protozoa which were tried in pure culture were able to decrease the number of bacteria in soil to a measurable extent, this might be accomplished through the combined action of all of them. Two experiments were performed in which a comparison was made of protozoa-free soil and of soil containing the six types of protozoa used in the preceding tests. For these tests the soils were allowed to incubate for two months as it might be contended that one month was not sufficient time to allow the "detrimental factor" to develop. These trials, in keeping with all of the tests which have been made with the individual organisms, gave wholly negative results.

TABLE XXV

Effect of mixture of protozoa upon the number of bacteria in soil

Test 1

POT	CONTROL	AVERAGE	PROTOZOA	AVERAGE
1	250,000,000	294,800,000	444,000,000	357,200,000
2	390,000,000		372,000,000	
3	270,000,000		384,000,000	
4	290,000,000		336,000,000	
5	274,000,000		250,000,000	

Test 2

1	180,000,000	193,300,000	180,000,000	176,000,000
2	240,000,000		176,000,000	
3	160,000,000		172,000,000	

The conclusion of this paper with bibliography will appear in the next number of the Journal of Bacteriology.

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A CULTURE MEDIUM FOR MAINTAINING STOCK CULTURES OF THE MENINGOCOCCUS[1]

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The maintenance of certain pathogenic bacteria upon artificial culture media is sometimes attended with great difficulty. Among these organisms the meningococcus may be said to occupy the first place. Its peculiar biology—particularly its intra-cellular ferment which is so potent a factor in its destruction—makes its viability at all times precarious. Furthermore its highly parasitic nature requires highly complex substances such as those upon which it grows in the human body.

While strains of the meningococcus that have been accustomed to artificial cultivation may be maintained upon plain nutrient agar, this medium is not favorable to its continued cultivation; the addition of glycerine offers no advantage; glucose results in more rapid growth and consequently more rapid degeneration. In their early work Councilman, Mallory and Wright used Loeffler's blood serum for both isolation and maintenance. Flexner used plain agar to which sheep serum was added. Some authors have used the serum of other animals—horse, goat, calf. Human serum and human ascitic fluid are conceded to be superior for isolation and for obtaining massive growth. The addition of the whole blood is possibly better than serum alone. Kutscher recommends a medium prepared with human placenta to which is added calf serum and glucose. For isolation Conradi used the centrifugalized spinal fluid, adding one part of the supernatant liquid to three parts of slightly alkaline nutrient agar; upon this solidified medium he planted the sediment.

[1]Presented at seventeenth annual meeting of the Society of American Bacteriologists, Urbana, Ill., December 28, 1915.

Fluid media offer no advantage over solid media and they are of course not adaptable for isolation. Gelatin is not suitable because the meningococcus does not grow at low temperatures.

While many of these media, offer satisfactory conditions for growth during a few generations, a fair proportion of strains kept upon them suddenly fail to develop and in spite of persistent effort cannot be resuscitated. Furthermore on all the above mentioned media the cultures under ordinary conditions must be transplanted at short intervals—not longer than two or three days—and kept constantly in the incubator at 37.5°C. (Exceptional strains are found which are unusually hardy and seem to require little more care than the common saprophytic bacteria.)

With all the above culture media, those favoring the rapid growth of the meningococcus at the same time result in rapid ferment production and consequently rapid death of the culture.

After innumerable trials we have found a medium which permits of relatively slow growth of the meningococcus with apparent suppression of ferment formation, thus resulting in greater viability. This medium has been in use for about two years and its superiority over the other media mentioned in the literature for the maintenance of stock cultures of the meningococcus seems to warrant its publication. This culture medium is a modification of the potato-blood-agar used by Bordet and Gengou for the isolation of *B. pertussis*.

Preparation of medium

1. Prepare potato extract as follows:

a. Potato peeled, cut in small pieces and washed in running water for about two hours, 100 grams.

b. Water containing 4 per cent double distilled glycerine free from acid, 200 cc.

c. Mix and autoclave for forty minutes.

d. Allow to stand over night and strain through cheese cloth.

2. Make potato-extract-agar as follows:

a. Mix in an Erlenmeyer flask: Potato extract, 50 cc.; NaCl solution 0.65 per cent, 150 cc.; agar 5 grams.

b. Heat in Arnold sterilizer until agar is melted, requiring from thirty minutes to one hour.

3. Tube without filtering, and sterilize, in autoclave for about forty minutes.

4. When wanted for use, melt the agar, cool to about 45°C. and add the desired amount of sterile defibrinated horse blood.

The amount of blood to be added depends upon whether or not the meningococcus has become accustomed to the medium.

In transplanting from another medium to this potato blood agar, a little difficulty may be experienced in getting the cultures started upon the new substratum. For this reason a large amount of the growth (not over twenty-four hours old) should be transferred to the medium containing about 20 per cent of defibrinated blood. In making the inoculation the culture should be rubbed slightly into the surface. This is incubated at 37.5°C. for about 2 days and then transplanted again to the potato-extract-blood-agar containing just sufficient blood to permit growth—that is, about 5 per cent. Subsequent transplants need not be made more often than every thirteen to fifteen days or longer, when kept at 37°C., provided that the cultures do not become too dry. In the case of cultures paraffined or sealed to prevent drying, a fair growth may be obtained after six weeks.

The meningococcus grows well at 37.5°C. At lower temperatures it will remain alive for a considerable length of time, although no growth occurs; viability may be retained at room temperature apparently as long as, if not longer than, at incubation temperature. Besides, maintenance at this temperature renders paraffining or sealing less imperative for the prevention of drying. Freshly transplanted cultures that were incubated for twenty-four hours at 37.5°C., and then kept at room temperature, showed fair growth after 4 weeks. Ice box temperature will kill most strains of the meningococcus in a comparatively short time. Cultures grown at 37.5°C. for twenty-four hours, and then transferred to the ice box, grew well after five days, but after ten days about twenty-five per cent failed to show any growth at all, 50 per cent showed scanty growth, and only 25 per cent a fair growth.

The meningococcus is an aerobic organism but like many other aerobes when first grown aerobically and then transferred to an atmosphere of hydrogen, it can be kept alive longer than when oxygen is present. Cultures of meningococci were grown aerobically for twenty-four hours at 37.5°C. and then transferred to a Novy jar, the air of which was replaced with hydrogen by means of a Kipp apparatus and a Schutte vacuum pump; the jar contained pyrogallic acid and sodium hydroxide which were permitted to mix after the air had been replaced several times by hydrogen. These cultures were then kept in the incubator; after ten weeks good growths were obtained on the first transplant.

The appearance of the meningococcus growth on potato blood agar is not very characteristic. After twenty-four hours growth at 37.5°C. the individual colony has reached the size of a small pinhead. It is gray in color, smooth and rather moist looking, of an amorphous consistency, the surface elevation varying from convex to pulvinate with border entire. With age, the color of the colony changes to dull gray, the consistency becomes tenacious, and the surface elevation more of the raised type.

Although the production of pigment by some organisms is facilitated on potato blood agar, the area of discoloration characteristic of numerous strains of streptococci,—notably the *Streptococcus viridans* and pneumococcus on blood agar, and some Gram negative cocci on glucose agar as described by Elser and Huntoon—has never been observed by us with any strain.

All of our thirty-eight strains have invariably remained Gram negative, regardless of culture medium used or age of culture. Occasionally, more frequently in old cultures, a few organisms may be seen that do resist for a time the action of the decolorizing agent and thus appear to be Gram positive. However, this is usually an indication of faulty technique or of contamination.

Arrangement in pairs is most common, although single cocci and groupings in tetrads are numerous, with certain strains especially. True chain formation has never been observed. Variations in the size of organisms in the different strains are

negligible, those of individual cocci seeming to be determined more by the age of the culture, than by the culture medium. Degeneration forms occur with all strains in quite young cultures.

The potato-blood-agar furthermore is of value for differentiating between the meningococcus and the gonococcus; on this medium the gonococcus grows only when the medium has a high blood and diminished salt content—the growth is always very scanty and the characteristic differences are immediately apparent.

BILE COMPARED WITH LACTOSE BOUILLON FOR DETERMINING THE PRESENCE OF B. COLI IN WATER

MAUD MASON OBST[1]

Irregular results have often been obtained in the past in the routine bacteriological examinations of water. The presumptive test for *B. coli* made with lactose peptone ox-bile upon a sample of water would give no indication of gas in 10 cc. quantities on one day, while another sample from the same source would show gas in 1 cc. or in 0.1 cc. quantities on the following day. It was observed that when bile which had been stored for six weeks at 1°C. was used for four samples of water from a polluted spring, and bile which had been freshly collected was used for six samples from the same spring, the former produced no gas, while the latter produced gas in 0.1 cc. quantities. Similar results were obtained with the use of bile which had apparently been carelessly collected.

In this laboratory it had been found necessary to obtain bile from an abattoir in a nearby city in 5-gallon lots, and to depend upon the workers in the abbatoir for its collection. When received at the laboratory, the bile was sterilized and stored at 1°C. until used, which was frequently for two months or longer. The above results showed that it must be collected more frequently and by one who would use proper precautions. This required one-half day's time every week of a reliable helper, and an expenditure of fifty (50) cents for three gallons. Even then, the helper met with serious difficulties in gaining access to that part of the abattoir in which he could secure the unbroken gall bladder.

[1] This work was carried on under the auspices of the Microbiological Laboratory, Bureau of Chemistry, Washington, D. C. The author desires to acknowledge the valuable assistance rendered by Dr. Charles Thom in the preparation of this paper.

These experiences led to communications with other bacteriologists,[2] all of whom expressed dissatisfaction with bile media. Prof. Edwin O. Jordan, University of Chicago, has stated, "Bile from different animals varies in composition, and this is probably one reason for the variable results obtained when this substance is added to culture media. Dried bile and bile salts have been used by various observers. In my own experience bile salts, like fresh bile, inhibited *B. coli* to some extent." Previous to the time when he made this statement, Jordan determined the "degree of inhibition" of *B. coli* by bile and reported that from one-third to one-half of the vital cells of *B. coli* were thus inhibited (Jordan, 1913). He stated also that there was no evidence that these cells were more attenuated or in any way less vigorous biologically than the others.

A résumé of the literature was then made, with regard to the origin of the use of bile as a medium. It was found that Jackson (1906) had experienced the difficulty of having *B. coli* inhibited by other organisms when he used glucose or lactose bouillon as recommended by Theobald Smith (1893, 1895). Jackson, therefore, experimented with MacConkey's bile salt agar, "Platner's Crystallized Bile," which consisted of a mixture of the two bile salts, and finally with his own medium which he made by adding 1 per cent lactose to fresh ox-bile, and which gave satisfactory results in his work.

Sawin (1907) corroborated Jackson in his recommendation of the use of lactose bile and regarded it as a satisfactory and delicate indicator of minor sewage pollutions of springs and wells.

When the necessity of finding a substitute for bile was recognized, dried bile was considered. This substance, being obtained from liquid bile, varies in composition approximately in the same manner as the original material. Biochemical laboratories overcome these variations to some extent by drying bile from large quantities of mixed liquid biles, but this precaution does not produce an entirely satisfactory product.

[2] Dr. F. L. Rector, Great Bear Spring Co., New York, N. Y.; Dr. W. W. Browne, College of the City of New York; Prof. S. C. Prescott, Massachusetts Institute of Technology; Prof. Earle B. Phelps, Hygienic Laboratory, Treasury Department.

The expense of obtaining bile salts discouraged their use, and it therefore seemed necessary to find a more uniform substance which could be easily obtained. In regard to glucose bouillon Weston and Tarbett (1907) have reported experiments upon the comparison of lactose bile and glucose bouillon in the examination of sixty-three samples of water, showing that although glucose permitted the production of gas from a larger number of samples than bile, yet *B. coli* could be confirmed from the glucose fermentation tubes in only 13 per cent of these samples. This result was confirmed by a small number of experiments conducted in this laboratory in 1911–1912.

Members of the Hygienic Laboratory of the U. S. Public Health Service have suggested the use of lactose bouillon and referred to satisfactory results which they have obtained from its use during the past few years. Their suggestion was strengthened by the report given by Theobald Smith (1895) that Chantemesse and Widal looked upon gas-production in lactose bouillon as conclusive evidence of the presence of *B. coli*, and by the work of Hall and Nicholls (1914) which showed that if the percentage of lactose added to bile be increased to 15 per cent the production of gas would be more rapid. Organisms of the *B. coli* group are the only aerobes[3] commonly found in water which will ferment lactose with the production of gas. The few anaerobes which might be found to produce gas may be eliminated by subsequent transfers to Endo's medium. It was, therefore, thought practical to substitute this medium for bile.

When a medium is used in large quantities, as bile usually is, comparative costs become important. Lactose and peptone are used in equal quantities in both media. The difference in cost of the two media depends upon the cost of the raw bile in one and the meat extract used in the other. Meat extract at contract price costs approximately three cents for the quantity required for making one liter of nutrient broth. The

[3] The Committee on the Standard Methods of Water Analysis, in their report read before the meeting of the American Public Health Association, held at Rochester, N. Y., in September, 1915, defined the B. coli group as including all aerobic bacteria which produce gas in lactose broth.

ox-bile requires one-half day each week of the time of the helper and fifty cents for three gallons for its collection, thereby costing approximately ten cents per liter of medium. Lactose broth can be made as desired from ingredients which do not materially change during storage.

In the experiments here reported check analyses were made upon a series of samples of water, using lactose peptone ox-bile and lactose broth. The bile was never used later than a week after collection. It was sterilized upon receipt and stored during this period at a temperature of 1°C. It was enriched with 1 per cent lactose and 1 per cent peptone, and tubed in Dunham tubes. The lactose bouillon was made from neutral nutrient broth prepared with 0.5 per cent Liebig's meat extract, 1 per cent peptone and 1 per cent lactose. This medium was compared with lactose peptone ox-bile in the examination of 191 samples of water with the following results:

No gas-producing organisms in 10 cc. quantities in either lactose or bile in 68 samples.

Gas-producing organisms in the same dilutions in lactose and in bile in 59 samples.

Gas-producing organisms in higher dilutions in bile than in lactose in 3 samples.

Gas-producing organisms in higher dilutions in lactose than in bile in 61 samples.

Lactose showed gas-producing organisms in one-half as much water as were shown by bile in 12 samples.

Lactose showed gas-producing organisms in one-fifth as much water as were shown by bile in 12 samples.

Lactose showed gas-producing organisms in one-tenth as much water as were shown by bile in 19 samples.

Lactose showed gas-producing organisms in one-hundredth as much water as were shown by bile in 5 samples.

From every sample *B. coli* was isolated from the highest dilution tube showing gas. This shows the ratio of inhibition of bile on *B. coli* when compared with lactose bouillon to be about 2:1.

In some instances only a small bubble of gas appeared in the inner tube in the bile fermentation tubes, while the production of gas in lactose filled two-thirds of the inner tube. In one instance a culture of *B. paratyphosus B.*, which showed characteristic agglutination in 1:500, was obtained from a lactose fermentation tube.

In order to obtain a direct comparison by total counts of the growth of the *B. coli* group on lactose and on bile collected at different times and stored for varying intervals, firm substrata were prepared by adding 1.5 per cent agar to each medium. It was found necessary to exert great care in filtering the bile agar in order that the final product should be free from a precipitate which would render the counting of the colonies difficult. The bile was collected each week, sterilized, and either made into agar at once or stored at 1°C. until used. For the tests, fifteen strains of *B. coli*, with characteristics described in the accompanying table, were grown for three days at 37°C., with daily transfers in 10 cc. nutrient broth.

In recording the results, it was found that the inhibition by the bile could be shown more clearly by taking the number of colonies which developed upon the bile agar as one, and considering the multiple of this number, which expressed the count on lactose agar from the same dilutious of the original culture plated and incubated at the same time, as the "ratio of inhibition" of the bile.

The bile which was collected during the month of June (see table) was tested with the cultures two days after receipt and again some weeks later. With a few exceptions the inhibition exerted by the fresh bile was less, in amount, and more regular for the different strains than that of the same bile after storage. When the actions of the various organisms upon the individual samples of bile are considered, it is seen that the variations in the degree of inhibition are greater for the bile that was held in storage before being tested; and, in general, this variation increased with the time of storage. One sample of bile (see table, (e)) was of a pronounced red color and contained a heavy red-brown precipitate. This sample showed an inhibitive action,

which was markedly irregular and which permitted no comparison with any other sample.

The contents of three individual gall bladders, collected on different dates, were also tested. The ratios of inhibition (see table, (f)) for any one organism on the three agars are nearly uniform and on the individual bile agar the different strains showed only a slight variation.

A comparison of the average inhibitive action of each sample of bile upon the different strains of organisms shows a variation from 2.4 to 3.8, and for the freshly collected bile a ratio of approximately 2.4 for all strains.

CONCLUSIONS

In these experiments lactose bouillon used as a substitute for lactose peptone ox-bile permitted the development of about twice as many *B. coli* from water as the bile medium.

Lactose bouillon costs less in money and labor. The difficulty of obtaining pure, fresh bile puts it almost out of the reach of many workers. The stored bile is proved to show progressive deterioration. Lactose bouillon can be prepared when desired and can be made more uniform. It need contain no precipitate to clog the inner tube or to affect the activity of the organism.

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Ratios of inhibition of B. coli on bile agar when compared with lactose agar*

CULTURE NUMBER	DESCRIPTION OF CULTURE	RATIOS OF INHIBITION BY BILE AGAR PREPARED FROM LIQUID BILE AFTER STORAGE AT 1°C. FOR													
		100 days	44 days	2 days (a)	42 days (a¹)	2 days (b)	34 days (b¹)	3 days (c)	27 days (c¹)	2 days (d)	20 days (d¹)	10 days (e)	2 days	2 days (f)	2 days (f)	2 days (f)
D-10	Typical acidi lactici	1.50	3.16	2.78		2.41	3.80	2.95	3.21	3.01	3.57	4.08	2.43	3.01	2.74	2.61
D-14	Slight gas in glucose, lactose, saccharose; none in dulcite; no indol	1.83	3.66	4.06	5.50	3.78	3.66	3.65	3.93	3.96	2.45	1.82	3.01	2.26	2.26	2.30
D-17	Gas in glucose, lactose, dulcite	1.65	1.47	2.11	3.55	2.32	3.38	2.41	2.78	2.37	2.48	2.48	2.40	2.45	2.30	2.21
D-18	Gas in glucose, lactose, dulcite (dulcite alkaline)	2.59	9.00	2.03	7.29	2.54	10.1	2.90	3.54	2.84	4.08	1.91	2.48	2.50	2.77	2.60
D-20	Gas in glucose, lactose; no indol	2.54	8.04	2.74	4.27	2.40	6.60	2.46	2.38	2.70	2.91	3.54	2.51	2.44	2.60	2.49
D-28	Gas in glucose, lactose, dulcite (saccharose after seventh day); no indol	2.01	2.69	2.37	2.90	2.50	2.91	2.90	2.94	3.22	3.72	1.98	2.32	2.22	2.19	2.30
O-1	Gas in glucose, lactose, saccharose (water)	2.40	2.57	2.57	3.01	2.40	2.99	2.34	2.87	2.43	2.65	3.20	2.60	2.44	2.61	2.50
O-2	Gas in glucose, lactose; none in saccharose (feces of white mouse)	2.31	2.65	2.18	3.08	2.21	2.94	2.30	2.54	2.20	2.59	4.15	2.30	2.31	2.40	2.38
O-3	Gas in glucose, lactose; none in saccharose (feces of rabbit)	3.95	1.84	2.50	5.86	2.54	2.66	2.40	2.61	2.45	2.67	2.50	2.51	1.97	2.14	1.98
O-4	Gas in glucose, lactose.; none in saccharose (feces of white rat)	2.72	2.50	2.46	2.30	1.50	1.82	2.44	2.55	2.80	2.37	1.19	2.30	2.20	2.10	2.30
O-5	Gas in glucose, lactose; none in saccharose; no indol; (water)	1.67	1.86	2.26	2.82	2.21	2.57	2.22	2.60	2.40	2.10	5.41	2.28	2.40	2.46	2.22
O-6	Gas in glucose, lactose; saccharose (cage of pigeon)	2.00		1.62	2.00	2.01	2.00	2.37	2.50	1.98	1.95	2.00	2.12	2.20	2.00	2.08
O-7	Gas in glucose lactose, saccharose (feces of dog)	3.89	4.48	2.51	3.18	2.00	2.61	2.21	2.48	2.20	2.70	4.41	2.20	2.71	2.64	2.54
D-Cream		2.74	2.16	3.01	4.95	2.45	3.30	2.72	3.12	2.50	2.72	3.81	2.45	2.47	2.50	2.50
D-Feces		7.53	3.46	3.09	4.33	2.91	4.02	2.74	2.81	2.81	3.50	3.21	2.48	2.54	2.50	2.31
Highest ratio		7.53	9.00	4.06	7.29	3.78	6.60	3.65	3.94	3.96	4.08	5.41	3.01	3.01	2.77	2.61
Lowest ratio		1.50	1.47	1.62	2.00	1.50	1.82	2.21	2.38	1.98	1.95	1.19	2.12	1.97	2.00	1.98
Average ratio		2.755	3.538	2.546	3.803	2.412	3.232	2.600	2.884	2.658	2.830	3.046	2.426	2.408	2.414	2.354

(a¹) Duplicate of (a).
(b¹) Duplicate of (b).
(c¹) Duplicate of (c).
(d¹) Duplicate of (d).
(e) Red in color, and contained a red-brown precipitate after storage.
(f) Contents of one gall bladder carefully collected.

* Obtained by making duplicate determinations on lactose agar and on bile agar in every instance and by dividing the total number of colonies developing on lactose agar after 2 days' incubation at 37° C. by the number developing on bile agar.

SOCIETY OF AMERICAN BACTERIOLOGISTS

Abstracts of Papers

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SYSTEMATIC BACTERIOLOGY

Under Supervision of S. H. Ayers

Studies on the Classification of the Colon-Typhoid Group. C.-E. A. Winslow and I. J. Kligler.

The committee on the classification of the colon-typhoid group, appointed at Philadelphia, has adopted a standard series of tests for titrable acidity, hydrogen ion concentration, milk reactions, indol production, gelatin liqefaction and chromogenesis. This preliminary report is based on the application of certain of these tests to 150 strains from the American Museum of Natural History collection; each strain being tested independently in New Haven and in New York.

Our results so far indicate that there are at least three major groups in the colon-typhoid series, the *B. coli* group clotting milk and producing a final high hydrogen ion concentration in glucose broth and forming indol; the *B. aerogenes* group, clotting milk but producing a final low hydrogen ion concentration in glucose broth and failing to form indol; and the *B. typhi* group giving a final alkalin reaction in milk but yielding a high hydrogen ion concentration in glucose broth and failing to form indol. The *B. coli* group includes at least three types, *B. communis* (indol positive, sucrose negative)—*B. communior* (indol positive, sucrose positive) and *B. acidi-lactici* (indol negative, sucrose variable). The *B. aerogenes* group is generally indol negative and sucrose positive and includes the gelatin liquefying *B. cloacae* as well as the typical *B. aerogenes*. The *B. typhi* group is indol negative and includes at least three types—*B. dysenteriae* (reaction in milk varying back and forth about the neutral point), *B. typhi* (initial acidity followed by alkalinity), and *B. paratyphi* (alkaline throughout).

The indol reaction as determined by the use of a tryptophane medium with 71 strains, gave results identical with those obtained by the use of peptone on the same strains two years ago. A positive Voges and Proskauer reaction is correlated with negative indol and a negative methyl red test for hydrogen ion concentration and clearly marks off *B. aerogenes* as a distinct group (the high gas ratio cultures of Rogers and Clark).

The Characteristics of Bacteria of the Colon Type Occurring in Human Feces. L. A. ROGERS, WM. MANSFIELD CLARK, AND H. A. LUBS.

A total of 113 cultures were obtained from 17 individuals and classified on the basis of carbon-dioxid hydrogen ratio, indol formation, gelatin liquefaction and the fermentation of certain carbohydrates and alcohols. All but 6 of the 113 cultures fermented glucose in the absence of oxygen with the production of almost exactly equal volumes of carbon dioxid and hydrogen.

This ratio agrees with that given by 99 per cent of the cultures obtained from bovine feces and differs radically from that given by nearly all of the grain cultures. Further agreement with the bovine feces cultures is seen in the high percentage of indol formers, the absence of gelatin liquefiers, a low percentage of carbohydrate fermenters but a relatively high ability in fermenting the alcohols.

The remaining six cultures produce nearly twice as much carbon dioxid as hydrogen and otherwise agree in a general way with the high ratio group which predominated in the grain cultures.

While this type occurred in relatively small numbers the actual number may amount to several hundred thousand in each gram of material. It is possible that the more frequent occurrence outside of the animal body of the high ratio type may be because it is more resistant to unfavorable conditions and consequently survives after the low ratio type has disappeared.

The Type of Colon Bacillus Occurring in Surface Waters. L. A. ROGERS.

A collection of 137 cultures of the colon type isolated from waters of greatly varying degrees of contamination were separated into two distinct groups. One of these included about one-third of the cultures and was evidently identical with the type which has been found to include 95 to 99 per cent of the colon bacilli of bovine and human feces. This type was found occasionally in springs in which there was no evident source of contamination but was especially abundant in rivers and streams usually considered to be polluted with sewage.

The second group which occurred in practically all waters examined was identified with a type which, while it responds to all of the usual tests for *B. coli*, occurs in feces in relatively small numbers. Cultures isolated from grains belonged almost exclusively to this type. The significance of this type in water cannot be determined but the characteristic fecal colon type can be demonstrated in polluted water with reasonable certainty.

Some Problems in Bacterial Nomenclature. R. E. BUCHANAN.

The following conclusions are reached:

1. A standing committee of the society, either the committee on classification or a new committee should be asked to consider problems of bacterial nomenclature and make suitable recommendations to the society.

2. Such a committee could study the international codes of nomenclature adopted by the botanists and zoologists and report such modifications as might be necessary to adapt them to the needs of bacteriologists.

3. They could recommend a suitable date of departure for bacterial nomenclature.

4. They could report upon the historical validity of the names that are used for bacterial groups, particularly genera.

5. They could prepare a list of recognized generic names.

6. They could seek the active cooperation of societies having the same interests, in this and other countries.

7. They could prepare resolutions to be presented at the next international botanical congress expressing the wishes of the society.

8. They could prepare a list of suitable names for the designation of chemical changes brought about by bacteria.

The Hemolytic Streptococci Found in Milk. Their Significance and their Relation to Streptococci of Human Origin. DAVID JOHN DAVIS.

The occurrence of epidemics of streptococcus sore throat having some relation to the milk supply has directed the attention of recent workers to the pathogenic properties of the various streptococci found in milk.

From supplies of milk obtained under various conditions including both pasteurized and certified milk a collection of strains was isolated and subjected to various tests. They were also compared with a collection of human hemolytic streptococci as regards their pathogenicity for animals.

Especial attention was given to the study of the property of heat resistance on account of its relation to pasteurization.

Only those streptococci were selected whose colonies were surrounded by a distinct clear zone of hemolysis on human blood agar plates (Type B, Theobald Smith).

The feebly hemolytic streptococci (type A) were often noted in the milk but were not collected and studied since the interest in sore throat epidemics has centered about the cocci with a clear wide zone.

Three hundred and twenty-eight sample specimens of bottled milk were collected from different dairies in the city of Chicago. Excepting 45 samples from one dairy which furnished certified milk all the specimens were pasteurized; and with the exception of two dairies (17 samples) the holding process was used.

The time of the year during which these examinations were made was from October 1914 to March 1915.

Blood agar (human) was used in plating and the counts were made after incubation at 37°C. at the end of 48 hours. The colonies of hemolytic streptococci were carefully noted and counted on the plates and later their identity was confirmed by further tests.

Eighty-five samples yielded on culture streptococci of the strongly hemolytic variety. The number in different samples varied consider-

ably ranging from a few hundred to several thousand per cubic centimeter. In the certified milk they were about in the same proportion as in the pasteurized samples. From one dairy in 16 specimens of milk no hemolysing streptococci were found. In all others some were found.

A study of these 85 strains of hemolysing streptococci was made as regards their morphology, cultural characteristics and certain other properties. They vary considerably among themselves. They are more resistant to heat than human strains of hemolytic streptococci. They possess little or no virulence for rabbits; therefore in all probability not for man. They rapidly acidify and coagulate milk and grow well at 20°C. They may form short or long chains but as seen in milk they often appear in pairs or a chain of few elements. While they are all definitely hemolytic (type B, Theobald Smith) the characteristics of the hemolytic zone on plates may vary in certain respects.

These milk strains are different from certain strains of hemolytic streptococci found at times in diseased udders in cows. These latter resemble the strains of hemolytic streptococci from human sources and are virulent for rabbits.

There is no reason to consider that these milk strains have any sanitary significance. The importance however of certain strains of hemolytic streptococci in relation to epidemics of sore throat makes it necessary to study carefully all such organisms in milk.

By itself the hemolytic property has no more value for identification purposes than many other characteristics and perhaps less than some. But it is of great importance on account of the practical value of the blood agar plate method as a means of initial separation of hemolytic strains from the many strains of non-hemolytic and feebly hemolytic streptococci found in milk.

Hydrogen-ion Concentrations in Cultures of Streptococci. S. Henry Ayers.

Hydrogen-ion concentrations were determined in cultures of streptococci from the following sources: 34 from various human infections, 18 from the human mouth, 89 from milk and 60 from the udder, feces and mouth of the cow, making a total of 201 cultures.

The cultures were grown in a broth containing 1 per cent cerevisine (a dry-yeast preparation), 1 per cent peptone, 1 per cent of the test substance, and distilled water. All the streptococci grew well in this medium and it does not contain a fermentable sugar. Many of the cultures would not grow in extract broth or in infusion broth when the muscle sugar was removed by fermentation with *B. coli*.

Since the acidity may be defined in terms of hydrogen-ion concentration this was determined instead of the titrable acidity. The test substances used were glucose, lactose, and cane sugar, raffinose, mannite and inulin, and the hydrogen-ion concentrations were determined by a colorimetric method.

The hydrogen-ion concentration can be represented by the symbol $P^{+}H$ and on this basis the neutral point of absolutely pure water is

P+H = 6.8 at 30°C. When P+H is less than 6.8, a solution is acid and when greater it is alkaline to pure water.

A study of a large number of cultures of streptococci seems to indicate that two limiting zones of hydrogen-ion concentration are reached, as may be seen in the table.

SOURCE OF STREPTOCOCCI	HYDROGEN-ION CONCENTRATION	
	$P^{+}H_{4.6-4.8}$	$P^{+}H_{5.5-6.0}$
34 from human infections	8	26
18 from human mouth	18	0
89 from milk	79	10
60 from udder, feces and mouth of cow	54	6

These hydrogen-ion concentrations have been found to be fairly constant and would probably be more definite if the values were determined electrometrically.

It seems evident from these results that streptococci reach more or less definite hydrogen-ion concentrations, which fact may serve to help in their classification.

The difference in the limiting hydrogen-ion concentration has not been correlated thus far with any other reactions, but it is rather striking that such a large percentage of the streptococci from cases of human infection reach only the lower zone of limiting hydrogen-ion concentration.

This difference among the streptococci, brought out by the hydrogen-ion concentration, can not be shown accurately by titration methods, since the titrable acidity depends upon the composition of the medium.

The Value of Lactose Bile for the B. Coli Presumptive Test. JOHN W. M. BUNKER.

The use of lactose bile media for the *B. coli* presumptive test has met with criticism because of the difficulty of obtaining fresh whole bile whenever and wherever needed. It has been maintained by some investigators that the use of lactose broth without bile is as efficient as the bile medium. Others have advanced the view that the inhibitive action of bile is so great that with its use weak forms of *B. coli* are lost.

The use of dried bile in the form known as Bacto Oxgall in 10 per cent solution has given results always as satisfactory as those obtained when preparing the medium from new whole bile, in tests upon oysters, milk, cream, ice cream, polluted water, and sewage.

When used in a 5 per cent solution dried bile has provided a medium which detects *B. coli* by fermentation in higher dilutions occasionally than when 10 per cent solution or Standard Lactose Bile is employed. The 5 per cent solution has never failed to show fermentation in equally high dilutions as the 10 per cent solution or the Standard medium.

A water to which was added a small quantity of *B. coli* culture when stored for a long time and tested daily with whole bile medium, ten

per cent dried bile, 5 per cent dried bile, 2 per cent dried bile, and lactose broth, showed the presence of *B. coli* at first in all media, later only in media containing 5 per cent or less of dried bile, and finally in lactose broth alone.

A water heavily polluted with feces tested in the same manner showed gas in one higher dilution in the 10 per cent dried bile and in whole bile than in the other media containing less amounts of ox gall. This water was so bad in appearance that a sanitary examination would have been unnecessary and therefore represents an exaggerated case.

For these somewhat contradictory results the following explanation is offered. It is believed that they are really not contradictory but are typical of the results which, being reported by different workers dealing with different material for test purposes, have confused the issue for some time.

The intestine is not filled with bile but bathed with it and the environment of intestinal organisms may be regarded as one of diluted bile. It seems reasonable then to suppose that a test tube of diluted bile offers a more favorable environment for these organisms than a test tube of whole bile. Organisms which develop in the standard medium do so in spite of the concentration of bile, and the weaker ones succumb.

The view is offered that in testing highly polluted material where the presence of organisms which will develop in the presence of lactose is so great that there is danger of overgrowth of *B. coli* and the shutting off of fermentation, the inhibiting property of bile toward non intestinal organisms is desirable.

Where the danger of overgrowth of *B. coli* is not great as in mildly polluted samples, lactose broth may be as efficient and will probably be more delicate.

The danger in using lactose broth is not that too many organisms will be included by the test, but that too many may be lost by overgrowth of others.

The question which ought to engage the attention of sanitary analysts is not "Shall we use bile or shall we use lactose?" but "How much bile plus how much lactose?"

The results of experimentation upon which this paper is based indicate that a medium composed of 1 per cent peptone, 1 per cent lactose, and from 2 to 5 per cent dried bile is much more delicate in detecting intestinal organisms by the fermentation test than is our present Standard medium, and is in the majority of cases more reliable than lactose broth alone.

A Chromogenic Bacillus. FRANK L. RECTOR.

This organism was isolated from water. It is a small rod with rounded ends, measuring from two to four microns long by seven-tenths of a micron wide. Occurs singly. Is motile. Stains evenly and easily; is Gram negative. Forms no nitrites, and indol production is doubtful. Coagulates milk in twenty-four hours. Forms gas in glucose, saccharose and glycerin; no gas in lactose. Thermal death point is 66°F. for ten minutes.

Produces a red pigment at room temperature. Rapidly liquefies gelatin and produces ammonia. Is a facultative anaerobe. Group number is 221.1012701.

On the Correlation of the Voges-Proskauer and the Methyl Red Reaction. MAX LEVINE.

The methyl red reaction of Clark and Lubs was suggested as a relatively quick and simple routine test for the differentiation of the colon-aerogenes group of bacteria. This test correlates strikingly with the accurately determined gas ratio. It is desirable that the methyl red reaction should also be correlated if possible with some previously employed test in order that the valuable work of the Department of Agriculture may be adequately compared with the numerous previous investigations.

From a study of 187 coli-like bacteria, 31 of which were obtained from other investigators and 156 isolated from raw and septic sewage and from the feces of the cow, pig, horse, sheep, and man, it was found that only those organisms which gave the Voges-Proskauer reaction were alkaline to methyl red. One hundred and fifty-nine of the organisms were negative to the Voges-Proskauer test; these were all acid to methyl red, while all of the 28 which reacted positively to the Voges-Proskauer test were alkaline to methyl red. It was also observed that although 23 per cent of the organisms isolated from sewage gave these two reactions (methyl red alkaline, V.P.+), no such organisms were isolated from the feces of the horse, cow, pig, sheep, and man.

The scarcity of organisms giving the Voges-Proskauer reaction in human feces is dwelt upon by MacConkey and also pointed out by Clemesha. Coli-like bacteria which give this reaction are characteristically of non-fecal origin. They resemble, in many other characters the *Bacillus aerogenes* (Escherich) and probably represent soil forms.

TECHNIQUE

UNDER SUPERVISION OF JEAN BROADHURST

Acidity of Media. H. A. NOYES.

Media for bacteriological purposes are standardized to definite degrees of acidity or alkalinity. There are several factors which may affect the accuracy with which the reaction of the media is determined. The paper, as presented, took up several of these factors and gave results of experimental work carried out in the Horticultural Research Chemistry and Bacteriology Laboratories of the Purdue Agricultural Experiment Station. The following conclusions were reached:

1. Agar-agar and gelatine should be the best obtainable. A grade of each, such that unfiltered solutions contain no visible solid matter, is obtainable.

2. Only necessary chemicals of the highest purity should be used.

3. The resulting products of reactions that will occur when a number of compounds are put together should be understood before one attempts to make combinations of them in a medium.

4. No indicator, used for the titration of media is accurate in all cases (even phenolphthalein which is very sensitive to acids will not indicate phosphoric acid accurately when it is in mixtures).

5. Enough media should be used for titration so that inaccuracies in measuring are minimized and errors in the actual titration will not be magnified in subsequent calculations.

6. The acid equivalent of media varies with the temperature of the media and the amount of variation is dependent upon the compounds present in the media.

7. Media should be added to carbon dioxide free distilled water and titrated at the temperature at which they are to be held when organisms are growing.

8. Distilled or double distilled water does not mean carbon dioxide free water. The carbon dioxide content of distilled water will not be a constant from day to day at a given laboratory.

9. Carbon dioxide in distilled water makes it possible to have a medium titrate, with phenolphthalein, 1.0 per cent acid[1] when it is alkaline or neutral.

10. The acidity of media that has been properly made does not increase appreciably when the length of time of sterilization is increased or when repeated sterilizations are made in the autoclave.

A Culture Medium for Maintaining Stock Cultures of the Meningococcus. C. G. A. Roos.

This medium has been in use for about two years and experience has demonstrated its superiority over the other media mentioned in the literature for the maintenance of stock cultures of the meningococcus. This culture medium is a modification of the potato blood agar used by Bordet and Gengou for the isolation of *B. pertussis*.

Preparation of Medium.

1. Prepare potato extract as follows:
   - *a.* Potato peeled, cut in small pieces and washed in running water for about 2 hours ..... 100 gm.
   - *b.* Water containing 4 per cent doubled distilled glycerine free from acid ..... 200 c.c.
   - *c.* Mix and autoclave for 40 minutes
   - *d.* Allow to stand over night and strain through cheese cloth.
2. Make Potato-Extract-Agar as follows:
   - *a.* Mix in an Erlenmeyer flask—
     - Potato extract ..... 50 c.c.
     - NaCl Sol. 0.65 per cent ..... 150 c.c.
     - Agar ..... 5 gm.
   - *b.* Heat in Arnold sterilizer until agar is melted, requiring from 30 min. to 1 hour.
3. Tube and sterilize without filtering in autoclave for about 40 minutes.
4. When wanted for use, melt the agar, cool back to about 45°C. and add the desired amount of defibrinated horse blood.

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[1] Requires 10 cc. of normal alkali per liter to make it neutral.

The amount of blood to be added depends upon whether or not the meningococcus has become accustomed to the medium. In transplanting from another medium to this potato blood agar, a little difficulty may be experienced in getting the cultures started upon the new substratum. For this reason a large amount of the growth (not over twenty-four hours old) should be transferred and about 20 per cent of blood be added to the medium. In making the inoculation the culture should be rubbed slightly into the surface. This is incubated at 37.5°C. for about two days and then transplanted again to the potato extract agar containing just sufficient blood to permit growth—that is, about 5 per cent. Subsequent transplantation need not be made more often than every thirteen to fifteen days or longer, when kept at 37°C., provided that the cultures do not become too dry. In the case of cultures paraffined or sealed to prevent drying, a fair growth may be obtained after six weeks.

Pasteurization Applied to Mold Spores. CHARLES THOM AND S. H. AYERS.

A series of experiments was devised to test the effects of temperatures commonly used in pasteurization upon the spores of pure cultures of a series of species of Penicillium, Aspergillus, Mucors, one form of Fusarium, and *Oidium lactis*. Results are summarized as follows:

1. The holder process of pasteurization in which milk was heated to 145°F. (62.8°C.), and maintained at that temperature for 30 minutes killed the conidia of every species investigated, except those of *Aspergillus repens*, *A. flavus*, and *A. fumigatus*. The molds which survive are only found occasionally in milk.

2. The flash process of pasteurization, where milk was heated to 165°F. (73.9°C.), for a period of 30 seconds, destroyed the spores of all the molds tested with the exception of many spores of one form and occasional spores of three more forms. At 175°F. (79.5°C.), only occasional spores of two forms developed.

3. When the heating process was performed in dry air for a period of 30 minutes at 200°F. (93.3°C.), 31 out of 42 forms of Penicillium and 7 out of 24 forms of Aspergillus were destroyed, but none of the cultures of Mucors. A temperature of 250°F. (121.1°C.) over a period of 30 minutes killed all the forms of Penicillium tried, but left an occasional living spore in one species of Aspergillus and 3 out of 6 Mucors.

The use of 0.01 Cubic Centimeter Pipettes in Bacterial Milk Analysis. JAMES D. BREW.

There are two common ways of measuring milk for counting bacteria with a microscope; one, by loops and the other by capillary pipettes. Prof. H. W. Conn, in his report on an investigation recently conducted in New York City, concludes that the wire loop as used by one of the laboratories making the counts appears to yield results as accurate as those secured by using 0.01 cc. pipettes. This conclusion is based upon comparative counts; but owing to possible wide variations in

microscopical counts, due to other factors than possible inaccuracy in measuring the quantity of milk under examination, a better means of testing is by comparing the weights of the amount of milk delivered. Twenty samples, weighed as discharged by one pipette, had a variation of 3 per cent, while under the same conditions the weights of the milk discharged from a loop varied 51 per cent.

Poorly formed tips and faulty calibration cause difficulty in securing the amount of milk desired. The tip should be a truncated cone with the flattened end a circle of about 2 mm. in diameter. Pipettes should be calibrated so as to deliver (not contain) 0.01 cc. of milk (about 0.0103 grams). This may be done by weighing the amount of milk discharged into the hollow of a clean, hollow ground microscope slide, covered to prevent evaporation. A pipette should deliver 4 to 5 per cent in excess of 0.01 cc. of mercury if this is used in calibrating, as mercury does not adhere to the walls of the pipette as do water and milk.

Pipettes should be clean, but sterilization is unnecessary. Sterilizing does not remove bacteria, and growth is stopped in quickly dried smears. Smears from ten samples of milk made by individual, sterile pipettes averaged 6150 bacteria per cubic centimeter. Smears made from the same samples by individual, clean pipettes averaged 4200 per cubic centimeter while those made from the same samples by one pipette which was recontaminated between each delivery in a high count milk and cleaned by rinsing in clean water, averaged 4600 per cubic centimeter. Smears made from the same samples by one pipette which was recontaminated between each delivery in a high count milk and rinsed in the sample of milk from which the next smear was to be made, averaged 338,900 per cubic centimeter while when smears were made in the same way but with the pipette unrinsed the average was 17,723,000 per cubic centimeter. These results indicate that for quantitative milk work, individual, sterile pipettes have no measurable advantage over individual, clean pipettes and that neither have any advantage over the use of one pipette for all samples, provided it is cleaned by rinsing in clean water. Dirty pipettes or pipettes handled carelessly. cause measurable errors.

A Simple Apparatus for Isolating Anaerobes. ZAE NORTHRUP.

A simple apparatus for isolating anaerobes consists in a 25 cc. burette complete with rubber tubing, glass tip and pinchcock. This is filled with any desired liquid nutrient medium, plugged with cotton, the delivery tube protected from contamination by inserting it in a small test tube and the whole apparatus set up on a ring stand and sterilized.

This tube may be inoculated with any material containing anaerobes. In a comparatively short incubation period the various classes of microorganisms will adjust themselves to their optimum oxygen requirements which will be noted sooner or later by a clear zone at the upper portion of the liquid varying in depth from a few millimeters to 8 or 9 cc. in some instances.

The organisms growing anaerobically may then be drawn off and sub-cultured further in these tubes until practically nothing but anaerobic organisms will be present. These may be isolated by the anaerobic plate method.

If successive sub-cultures are to be made from the same tube it is necessary to have the rubber tubing on the burette several centimeters in length so that a pinchcock may be placed about 3 cm. above the original one, the rubber tubing cut with sterile scissors and a sterile burette tip inserted. This is necessary as after one withdrawal of liquid, aerobic organisms may grow in the liquid remaining in the tip.

Modification in Staining Technic. ZAE NORTHRUP.

The following modification in the technic of Gram's stain is recommended for beginning students in bacteriological laboratory work. If a coverglass preparation of two morphologically different organisms, one gram positive, the other gram negative, is made on the same coverglass and stained by Gram's method, the student is enabled to get the differentiation very clearly. *B. coli* and *B. subtilis* lend themselves to this differentiation very nicely.

Amniotic Fluid as a Bacterial Culture Medium. WARD GILTNER AND L. C. LUDLUM.

Amniotic fluid is a normal transudate from the blood of the pregnant mammalian female. It is a very dilute albuminous solution containing various salts and also cellular elements in suspension. While its composition varies with the different species of animals and with the stage of pregnancy it was believed that its composition was sufficiently constant and quite probably, qualitatively and quantitatively, in conformity with the food requirements of many parasitic bacteria. In fact it was believed that on account of the ease of collection and the large quantities available, bovine amniotic fluid would serve as a substitute for, or perhaps have advantages over ascitic fluid or blood serum media. At all events amniotic fluid need not be collected aseptically since it can be sterilized either in the autoclave or by Tyndall's method.

In our experiments amniotic fluid has been used in place of broth with no additions, and with agar and gelatin or with glycerine. With the colon-typhoid group, *B. coli* gave excellent growths, *B. typhi* grew scantily and *B. cholerae-suis* only fairly well. *Staph. aureus* grew best on glucose agar and on glycerine amniotic agar. None of the strains of *Streptococcus pyogenes* studied grew well except in bouillon. The growth was least on the solid amniotic agar which is a fact of considerable interest since, in the few tests conducted, *B. diphtheriae* from swabs could be isolated readily on the amniotic agar. The isolation of new strains of *B. diphtheriae* on amniotic agar was facilitated by the inhibiting action on streptococci. Involution forms of *B. diphtheriae* were not so abundant on amniotic agar as on Loeffler's blood serum medium. An old culture of *B. tuberculosis* grew with

marked vigor. For a number of years we have been satisfied that plain amniotic agar was a very nearly ideal medium for *B. abortus* (Bang).

Study of Effect of Dilution Water on Bacterial Suspensions. H. M. WEETER.

A series of tests were made to determine the quantitative changes in bacterial suspensions held in dilution water. From suspensions held for two hours at room temperature triplicate plates on lactose agar were made at fifteen minute intervals.

Of fourteen tests made with dilutions of one part milk in one hundred thousand parts water, five showed decreases ranging from ten to seventy per cent in two hours, seven showed no definite change, and two gave increases of thirty-two and thirty-nine per cent.

Since colonies of the lactic acid type seemed to be the ones disappearing thirty-two tests were made with milk inoculated with these organisms in dilution water from five different sources. The same dilution was used as before. Thirty of these tests gave unmistakable reductions in numbers, amounting in some cases to fifty per cent in fifteen minutes.

Additional tests of dilutions below one to one thousand made with milk containing a mixed flora did not show any decrease during two hours, but an increase was observed on longer standing.

The nature of the organisms present and the amount of material added to the dilution water from the original bacterial medium are probable factors determining the effect of dilution water on the bacteria present.

Variation in Plate Counts Under Research Conditions. M. J. PRUCHA.

Results of seven experiments are presented in this preliminary report. In each experiment about one hundred lactose agar plates were prepared using the same dilution of milk.

Further study is needed to give sufficient basis for drawing definite conclusions, but the results so far point to the conclusion that the average of three plates from the same dilution approaches reasonably closely to the average of a hundred plates made from the same dilution, when that average is between one and two hundred colonies per plate.

Another Use of the Double-Plate Method. W. D. FROST AND FREDA M. BACHMANN.

This method was used by Frost in 1904 to study antagonism among bacteria. It was slightly modified, renamed and used by Churchman in 1912 to study the bactericidal action of aniline dyes.

It is here modified to obviate the necessity of using either a glass or metal division by putting in a petri dish, before sterilization, a semi-disc of cheesecloth. The bottom of the dish is entirely flooded with the medium to be used and when hard the piece of cloth with the adherent agar is lifted out with sterile forceps. The clear portion of the dish is flooded with the medium containing the material to be studied. Streaks of the organism to be used are then made over each side of the

dish. In this way the agar on both sides of the dish is in perfect contact which makes diffusion readily possible.

These plates are being used to study the effect of spices and condiments in inhibiting yeasts, molds, and bacteria. This method serves as a satisfactory means of determining the preserving action of these substances.

INDUSTRIAL BACTERIOLOGY

Under Supervision of D. H. Jones

A Possible Function of Actinomycetes in soil. H. Joel Conn.

A comparison has been made at the New York Experiment Station between the number of Actinomycetes in sod and in cultivated soils. The samples have been taken in pairs, one from sod and one from cultivated soil, the two spots selected always being within a few yards of each other. Thirty-five pairs of samples have been taken; and a considerable variety of soil types have been sampled. The comparative counts have been made by means of gelatin plate cultures.

Sod soil, almost invariably, has given a higher count of Actinomycetes than cultivated soil; also the Actinomycetes have comprised a greater proportion of the total flora in sod soil than in cultivated soil. In the thirty-five samples of cultivated soil, Actinomycetes comprised, on the average, 20.5 per cent of the total flora; in the samples of sod soil, 37.5 per cent, or almost twice as high a proportion. There were only three or four exceptions in the whole series, and in those the difference in favor of cultivated soil were so small as to be insignificant. The differences in favor of sod soil on the other hand have never been negligible, and sometimes have been extreme—as in one case where Actinomycetes comprised only 2 per cent of the flora in the cultivated sample but 16 per cent in the corresponding sod sample, or in another where they comprised 15 per cent of the flora in the cultivated sample, but actually as much as 60 per cent of the flora in the corresponding sod sample.

A further series of tests comparing three spots in a single soil type, one fallow, one in new sod, and one in old sod, showed 10–15 per cent Actinomycetes in the fallow spot, 21 to 25 per cent in the new sod and 37 to 39 per cent in old sod.

The most probable explanation of this difference seems to be that the Actinomycetes are active in the decomposition of grass roots, a point which is now being investigated at the New York Experiment Station.

Media for Soil Bacteria. H. A. Noyes.

In the course of other investigations in the Horticultural Research Chemistry and Bacteriology Laboratories of the Purdue Agricultural Experiment Station it was necessary to select a medium for the plating of soil organisms. The media first chosen for comparison were the Lipman and Brown agar, H. J. Conn's sodium asparaginate agar, and soil extract agars. Agar-agar alone was also used in comparison with the above media.

In plating tests the Lipman and Brown agar proved to be the best of the media studied, not only from colony counts, but in the variety of organisms grown. This latter is judged from the macroscopic appearance of the plates and by the fact that some organisms growing on the Lipman and Brown agar would not live when transferred to other media.

The colonies on the sodium asparaginate agar were large, but in only one of over fifty tests of organisms growing well on this media did the organisms fail to grow on agar alone.

The two best media were so different in constitution and the organisms that grew best on them acted so differently when transferred from one medium to the other that those ingredients furnishing carbon and nitrogen were investigated further. These tests resulted in other tests where 15 gms. of (best obtainable) agar-agar was the basis of all the media. This was used both alone, and in combination with ammonium nitrate, starch, Witte's peptone, sodium asparaginate, Liebig's extract of beef; soil extracts, wheat straw extracts, and leaf extracts were also used.

The results of this work and of later work are shown in the following table. The soil organisms reported on were chosen because of their macroscopic differences. The known pathogens and known non-pathogens were the only known organisms studied.

Media tests

(15 grams agar-agar is basis of all media.)

MEDIA	24 SOIL ORGANISMS		7 KNOWN PATHOGENS AND 8 KNOWN NON-PATHOGENS					
			Rank 3 days		Rank 6 days		Rank 8 days	
	Rank 5 days	Rank 13 days	Pathogens	Non-Pathogens	Pathogens	Non-Pathogens	Pathogens	Non-Pathogens
Lipman plus Brown	3	4	12, 13	7	12	7	11	9
0.05 gram Witte Peptone	15	15	17	16	16	16	16	16, 17
2 grams St. plus 0.05 Peptone	6	7	10, 11	13	9	12	9	12, 13
H. J. Conn's media	8	9	6	8, 9, 10	6	8	6	7
1 gram Na. asparaginate	14	14	16	14	15	13, 14	14	14
2 grams St. and 1 gram Na. asp.	9	12	8	8, 9, 10	7	9	7, 8	8
Soil plus 1 gram NH_4NO_3	16	16	15	17	17	17	17	16, 17
Soil plus 1 gram Starch	13	13	14	15	10	15	13	15
Soil plus 2 grams Starch	10	8	10, 11	8, 9, 10	11	13, 14	12	13
1 gram NH_4NO_3 plus 1 gram Starch	7	6						
1 gram Starch	11	10	9	11	13	11	10	10
2 grams Starch	4	3	5	6	4	5	5	5
7.5 grams Gelatine	12	11	12, 13	12	14	10	15	11
7.5 grams Gel. plus 1 gram Starch	5	5	7	5	8	6	7, 8	6
7.5 grams Gel. plus 2 grams Starch	2	2	4	4	5	4	4	4
7.5 grams Gel. plus 2 grams Starch plus soil	1	1	3	3	3	3	3	3
5 grams Liebig's Ext. plus 10 grams Peptone			2	2	2	2	2	2
5 grams Liebig's Ext. plus 4 grams Starch			1	1	1	1	1	1

Are Spore-Forming Bacteria of any Significance in Soil Under Normal Conditions? H. JOEL CONN.

A series of tests has been made at the New York Experiment Station to determine whether *B. mycoides*, *B. cereus* and *B. megatherium*, the most common spore-forming bacteria in soil, occur under normal conditions as spores or as vegetative rods. Diluted soil infusion was heated at a temperature of 75 to 85°C., heated and unheated samples of the infusion plated, and the colonies of these three types on the two sets of plates counted. It was assumed that the colonies on the plates from heated infusion represented spores and that the difference in favor of the unheated infusion, if any, represented the vegetative forms.

In a series of 22 tests the average count of all three spore-formers was: 775,000 per gram, unheated; 726,000 per gram, heated. They were higher 14 times in unheated infusions, 8 times in heated. The greatest difference in favor of the unheated infusion was 530,000 per gram, which was offset by a difference of 450,000 in favor of the heated infusion; a fact which suggests that both were within the limits of experimental error. Considering the organisms separately:

B. mycoides was higher 12 times in unheated infusions, 8 times in heated.

B. cereus was higher 10 times in unheated infusions, 8 times in heated.

B. megatherium was higher 12 times in unheated infusions, 8 times in heated.

The number of spores or the total number of spore-formers present did not increase even in a pot of soil mixed with a heavy application of fresh horse manure.

These figures suggest that bacteria of this group normally occur in soil only as spores, in which form they cannot be active. This is surprising, as they are universally present, and have always been considered important. What their actual significance may be is a question.

Ferrification in Soils. P. E. BROWN AND G. E. CORSON.

Preliminary studies of the oxidation of iron in the soil, or ferrification, have shown that the process is rather a complicated one. An attempt was made to ascertain whether soils have a ferrifying power, whether such a power is bacterial or chemical in nature or the result of a combined action of several groups of factors, whether different soils have varying ferrifying powers, whether ferrification can be measured in the laboratory and finally whether the process is of any significance from the soil fertility standpoint.

Much difficulty was experienced in devising methods for the work, owing to unsatisfactory chemical methods and many series of tests were carried out merely to solve some of the chemical problems involved. A method has been devised which, although still rather crude, will permit of tentative conclusions.

Soils from a wide variety of sources were tested and it appears that ferrification is a process of common occurrence in the soil, that different

soils possess differing ferrifying powers, and that the process is in part chemical and in part bacterial in nature. A line of investigations is thus opened up which may prove of much interest. Ferrification in soils apparently should be studied further both from the technical and from the practical standpoints.

Coli-like Organisms of the Soil. B. R. JOHNSON.

Forty-two samples of soil were examined for the presence of coli-like bacteria. Eighteen samples were from manured and twenty-four from unmanured areas. It seems from this study that in both manured and unmanured soils, the incidence of coli-like organisms is considerably greater, if a crop is being raised than if the soil is fallow.

In a preliminary study of 363 cultures as to their reaction with methyl red and the Voges Proskauer test, 261 were found to be alkaline. Of these over 84 per cent reacted positively to the Voges Proskauer test. Of the 219 cultures which gave the Voges and Proskauer reaction, over 97 per cent were alkaline to methyl red. This striking correlation between the two reactions has been previously pointed out by Levine, who observes that such forms are rarely found in feces, but relatively abundant in sewage. Rogers and his co-workers found methyl red alkaline coli to be the prevailing type on grains and seldom present in cow manure. The prevailing coli-like organisms in the soil are apparently of a non fecal type and they may be differentiated from fecal strains by the Voges Proskauer and methyl red reactions.

The Influence of Soil Solution on the Longevity of Microorganisms Subjected to Desiccation. WARD GILTNER AND VIRGINIA LANGWORTHY.

It is already well known that bacteria resist desiccation in soil for a much longer time than in a naked or unprotected condition such as might be offered by the surface of a solid culture medium. It is also known that any porous substance, not in itself antimicrobial, will offer protection to microbes against the deleterious effects of desiccation. There is a question as to just what factors are responsible for the prolonged life of microbes in the soil. Soil solution, as used in these tests, was extracted by the paraffin oil displacement or pressure method. Tests were made with other solutions, viz., physiological salt, 0.1 per cent agar, gelatin, albumin, gum arabic, soluble starch, also nutrient broth and milk using *Ps. radicicola* and drying in quartz sand after suspension in the different solutions. The results were that: (a) after suspension in normal salt, gum arabic, starch or agar solutions drying in sand was rapidly fatal, few or no bacteria being alive after one month, (b) after suspension in gelatin or albumin solution drying was less rapidly fatal, (c) after suspension in milk or bouillon drying in sand was still less rapidly fatal. Suspension in soil solution followed by drying in sand gave in one case better results than with milk and all other solutions used except broth and in another case better than all other solutions except milk and broth. Tests were also made of the longevity of *Ps. radicicola* dried in quartz sand and in clay loam.

Further tests were made to determine the changes in numbers and kinds of microorganisms naturally occurring in soil solution when it is dried in different types of soils, sand, sandy loam, clay, clay loam and muck, using soil solution from a rich garden loam.

Conclusions from all the experiments were that:

1. The survival of nonspore-bearing bacteria in air-dry soil is due, in part, to the retention by the soil, of moisture in the hygroscopic form. This, however, is not the only factor, for the longevity of bacteria in a soil is not directly proportional to its grain-size and hygroscopic moisture.

2. Bacteria, at least those tested, resist desiccation longer in a rich clay loam than in sand under the conditions of our experiment.

3. The solution extracted from a rich clay loam contains substances which have a protective influence upon bacteria subjected to desiccation.

Reaction of the Soil Solution as an Index of Biological Changes in the Soil. J. F. MORGAN AND O. M. GRUZIT.

One of the essential problems in the study of soil fertility is to adjust the reaction of the soil. This reaction essentially influences the chemical, the physical conditions and the biological life of the soil.

The junior author has found in his preliminary study of the soil solution adjusted to various degrees of reaction with N/100 mineral acid and N/100 alkali and mixed with pure sterile quartz sand, that this reaction had some effects upon the number and the type of bacteria.

An acid reaction of N/1200 had a distinct toxic action on the growth of the bacteria and the most suitable reaction for the growth of the soil bacteria was in the neighborhood of N/1000 alkali.

When changes occur from alkaline to neutral and to acid, the numbers of bacteria increase up to the point where the solution is barely alkaline and then, decrease after this point is passed. In cultures with an acid reaction, the lowering of this acidity causes the soil bacteria to increase rapidly.

The Soil Solution as an Index of the Biological Changes in the Soil. J. FRANKLIN MORGAN.

The soil solution is a homogeneous mixture of the soil water and the soluble soil constituents, both mineral and organic.

The soil solution offers a good medium for the study of some of the biological changes in the soil. As it is the work shop of the microorganisms, this solution will contain the products of their work.

In some nitrogenous experiments with dried blood, tankage, and cotton seed meal, marked changes in the forms of nitrogen and physical conditions of the solution were noted at the different periods of extractions.

The longer periods showed a decrease in ammoniacal-N and an increase in nitrate-N. In all cases there was an increase in the total solids. This had its effect upon the physical conditions of the solution, e.g., specific gravity, specific conductance and similar phases.

The Indirect Effect of Certain Soil Treatments Upon Bacterial Activity. P. L. GAINEY.

Different methods of preparing seed beds for winter wheat at the Kansas Station have given very large differences in the accumulation of nitrate nitrogen prior to seeding. Early (July 15) versus later, and deep (eight inches) versus more shallow plowing, have given higher nitrate contents. Efforts to trace observed differences to variations in bacterial flora have failed. Evidence was presented showing a correlation between moisture content of the surface soil and nitrate accumulation. A summary of data accumulated indicates that the various treatments have had but slight effect upon the organisms concerned in nitrate formation, except in so far as this activity is controlled by other factors.

Studies on Soil Protozoa and their Relation to the Bacterial Flora. J. M. SHERMAN.

The occurrence and activity of protozoa in soil. The results obtained from sixteen fertile soils representing various soil types indicate that these soils contain about 10,000 protozoa per gram. The predominating protozoa in the soils studied were flagellates. Ciliates and amoebae were occasionally found in numbers approximating 1,000 per gram.

It was demonstrated that certain types of flagellates are capable of multiplication in soil. The ciliates which were tested were not able to increase in soil when kept at its normal moisture content.

The effect of protozoa upon the soil bacteria. Observations made on soils containing protozoa and free of protozoa, at various temperatures, with different moisture contents, and on various types of soil indicated that the protozoa in the soils studied did not have a limiting action upon the bacterial flora.

The effect of specific types of protozoa, in animal-pure cultures, upon the soil bacteria was also studied. The ciliates limit the development of bacteria markedly in soil extract, but are not able to exert this effect in soil, since they do not lead a trophic existence under ordinary soil conditions.

Of four types of active soil flagellates which were tested three had no apparent effect upon the number of bacteria, either in soil or in soil extract. The fourth organism, a species of *Monas*, had a marked limiting action upon the bacterial flora in soil extract but apparently had no effect in soil.

The effect of volatile antiseptics upon the soil micro-organisms. The treatment of soil with volatile antiseptic does not free it of protozoa. The active soil protozoa again multiply and attain their normal numbers within one month after treatment.

The maximum numbers of bacteria in partially sterilized soils are not found while the protozoa are suppressed, but after these organisms have again reached their maximum numbers.

The number of bacteria in treated soils cannot be decreased by reinoculation with one per cent of untreated soil.

Comparisons made of treated and untreated soils under various conditions failed entirely to give any evidence in support of the theory that there exists in soil a harmful biological factor which is destroyed by the action of volatile antiseptics.

The Relation of Protozoa to Certain Groups of Soil Bacteria. T. L. HILLS.

In this work studies were made of the effect of protozoa on ammonification, nitrification and free nitrogen fixation in soil.

Ammonification. Three sets of the same sandy loam soil were used. One set was left untreated, another heated to 90°C. for one hour and the third heated as above and later inoculated with 1 per cent of the original untreated soil thus introducing the supposed harmful factor, the protozoa. The ammonia and nitrate determinations after 30 days revealed the following: in the untreated soil the ammonia content remained about the same while the nitrate increased slightly; in the heated soil the ammonia increased considerably but the nitrate remained the same, as the nitrifiers had been destroyed by heating the soil. In the heated and reinoculated soil the ammonia decreased slightly but there was a decided increase in nitrate formation. The protozoa introduced did not seem to have any detrimental effect on the production of ammonia and its subsequent oxidation to nitrate.

Nitrification. Flasks containing soil were sterilized by heating at 15 pounds pressure for two hours. Then one half of them were inoculated with a suspension of normal soil in sterile distilled water and the remaining half were inoculated with the same amount of protozoa free soil. This latter soil was obtained by sterilizing soil and inoculating it with as many different kinds of bacteria as could be isolated by the plate method. All flasks were then inoculated with protozoa free cultures of Nitrosomonas and Nitrobacter. Then a definite amount of ammonium sulphate was added. The ammonia and nitrate were determined after 28 days' incubation. There was but very little difference in the rate of conversion of ammonia to nitrate in the two different soil cultures.

Free nitrogen fixation. Sterile soil cultures were inoculated one half with normal soil and the remainder with protozoa free soil. Then a suspension of Azotobacter (and 1 per cent mannite in sterile solution) was added and after incubation of 21 days at 25°C. the cultures did not show any appreciable difference as regards the amount of nitrogen fixed, the difference being quite well within the limit of experimental error. In liquid cultures sterile Ashby's solution was used, one set being inoculated with soil containing protozoa and the other set with soil free from those organisms. Suspensions of Azotobacter were also added. After 21 days' incubation at 25°C. the total nitrogen analyses revealed a noticeable difference in free nitrogen fixation; those cultures free from protozoa fixing 2.05 mgs. as an average per 100 cc. of solution in excess of those containing protozoa. It seems evident that the protozoa finding here a medium suitable for their development destroyed many of the Azotobacter cells.

In conclusion it would seem from the experiments above cited that the protozoa do not have a detrimental effect on the processes of ammonification, nitrification and free nitrogen fixation in the soil.

A Study of the Nodule-Forming Bacteria. F. O. OCKERBLAD.

This paper deals with the relative longevity of *Ps. radicicola* (twelve strains from the more common legumes) in sealed and unsealed culture bottles such as are used for distribution of nodule-forming bacteria. The media used are a liquid medium (1000 cc. ash leachings from 5 grams wood ashes plus 1 per cent saccharose) and a solid medium (same as above plus 1 per cent agar).

In preparation all bottles were inoculated and incubated at room temperature (20°–22°C.) for two weeks, then half the number of each of the solid and liquid cultures of the different strains were sealed by removing the cotton plug and inserting cork stoppers which had been soaked in mercuric chlorid 1: 1000 and flamed at time of insertion. At 10-day intervals a culture of each strain on both the solid and liquid media is analyzed by plating in ash agar to enumerate the number of living cells and by making direct count with Thoma counting chamber for total numbers, dead and alive.

The bacteria in the liquid cultures both the sealed and unsealed are dying quite rapidly, with the greatest rapidity in the sealed cultures. On the solid medium the number of living cells in the sealed cultures is decreasing, approximately 25 per cent in 20 days; while the unsealed are showing little or no decrease. The total number of bacteria on the solid medium is greater than in the liquid culture media.

If we may be permitted to draw conclusions from limited and incomplete data we should say that a liquid medium for the distribution of nodule-forming bacteria is unsuitable because of the small total number and of a high mortality; and that the deterioration of cultures on solid medium in sealed form should be recognized.

Quantitative Media for the Estimation of Bacteria in Soils. R. C. COOK.

Comparative tests of several different media upon twenty soils are reported.

The length of incubation period and manner of sterilization were incidentally studied as affecting the comparative values of the respective media with the indication that five days is of sufficient duration to secure satisfactory counts.

Lipman and Brown's modified synthetic agar, Temple's peptone agar, Brown's albumen agar, and Conn's sodium asparaginate agar were compared with several other agar media having varied sources of nitrogen.

Highest counts were obtained quite consistently with the sodium asparaginate agar during the first part of the work. Later a medium was developed in which ammonium nitrate and urea were employed; this gave results fully as good as any other in a limited number of tests. Albumen agar in which the albumen was dissolved in sodium hydroxide

instead of water gave much more consistent counts, and in most cases followed closely the sodium asparaginate agar, surpassing it in one or two instances. Molds quite frequently affected the ease in counting in the albumen agar, but less so in the ammonium nitrate-urea agar. In the case of the asparaginate agar, however, there was no difficulty experienced from this cause.

It was also observed that inasmuch as all soils do not behave in the same manner toward the different media it is essential to use several soils in the comparisons; otherwise misleading results may be obtained.

Differences between the counts on the various media were not as large as might be expected and there seems to be no justification for the belief that any particular one will be most satisfactory in all cases.

Bacteria, Actinomyces, and Fungi in Soils. SELMAN A. WAKSMAN.

This investigation has been undertaken with a view to demonstrate the relationship between these three groups of microorganisms in different soils and at different depths. Soils of different texture and structure were used, and samples taken at six different depths. A six-day incubation period has been used for the counts of the bacteria and fungi and a fourteen-day period for the actinomyces counts. The results indicate that soils rich in bacteria are also rich in fungi and actinomyces. The largest numbers of all the three groups occur within the upper eight inches of the surface soil. The bacteria decrease regularly with depth, in numbers and also in percentages relative to the total numbers of microorganisms. The numbers of fungi decrease also with depth and they almost disappear below eight to twelve inches. The actinomyces numbers decrease with depth, but below eight to twelve inches their numbers remain constant up to thirty inches, and their percentage relative to the total numbers of microorganisms increases regularly with depth, because the bacterial numbers decrease, and fungi almost disappear. At a depth of 1 inch the bacteria form 81 to 86.5 per cent, fungi 6.2 to 7.1 per cent, and actinomyces 7.3 to 12.1 per cent; at 30 inches the bacteria form 16.4 to 42.1 per cent, fungi 0 to 5.6 per cent, and actinomyces 52.7 to 83.6 per cent of the total microorganic flora of the soil developing on agar plates. The actinomyces form a numerous group of soil microorganisms, especially in the lower soil depths; over 30 species of them have been isolated. The following groups of fungi occur in the soil in the largest numbers: Penicillia, Mucors, Aspergilli, Cladosporia, Trichodermae, Fusaria, and Alternaria. Many more fungus types have been isolated, but their numbers are limited.

FOOD

UNDER SUPERVISION OF CHARLES THOM

Comparison of the Number of Water Bacteria Growing on agar at 37°C. and on Gelatin at 20°C. FRED W. TANNER.

The recommendation of the Committee on Standard Methods for the Examination of Water and Sewage of the American Public Health

Association in their 1912 report, that the colony count on agar be adopted as the standard, has not met with the approval of many bacteriologists. In order to secure more data on this subject the Illinois State Water Survey began a series of comparative tests. A large number of analyses was made of which 4379 are considered in this paper.

In order to reach a more definite basis for comparison the analyses were arranged in the following classes according to their sources. The table indicates the ratios which were found. In each case the agar count was taken as unity.

SOURCE	NO. OF SAMPLES	AGAR GREATER THAN GELATIN	AVERAGE RATIO	MAXIMUM RATIO	GELATIN GREATER THAN AGAR	AVERAGE RATIO	MAXIMUM RATIO
Deep wells	712	54	1.1–1	25–1	658	1–1.4	1–76
Shallow wells	1648	148	1.6–1	17–1	1500	1–1.4	1–68
Raw Lake Michigan water	405	3	156–1	466–1	402	1–16	1–633
Raw river water	537	56	1.4–1	4.1–1	481	1–9.0	1–250
Treated water	1077	151	1.9–1	12–1	926	1.1–8	1–35

The relation of agar colonies to gelatin colonies on those samples showing the larger number on agar does not exceed 10 to 1, with one exception and this exception results from the consideration of only three analyses. With those samples showing the greater number on gelatin, the ratio does not exceed 1 to 10, except in one case and this again is on raw Lake Michigan water. This would seem to indicate that in badly polluted waters we might expect a high ratio, but with pure waters the counts on the two media closely approach each other.

Scientific Methods of Control in the Mineral Water Industry. FRANK L. RECTOR.

Methods of protecting the source and handling the product of the Great Bear Spring Company from the spring to the consumer are discussed. This company owns 600 acres of land comprising the entire watershed of a group of springs, 11 in number, whose flow is about one-half million gallons daily. The springs are situated five miles south of Fulton, New York.

Some 360,000 evergreen trees have been used to reforest this tract of land. Water from three of the springs is used. The springs are enclosed in enamel or glass-lined steel caissons with light-proof covers. They are perfectly protected from surface drainage.

The water is shipped in large tanks, also glass and enamel-lined, of a capacity of from seven to ten thousand gallons. Cars are sterilized by steam at 10 pounds pressure for one hour before filling. After

filling, each car is given a twenty-four hour test for gas production before being shipped.

Upon arrival at destination the car is connected by a metal hose to the storage tanks in the building and the line sterilized with steam before the car is emptied. The storage tanks are glass lined and are sterilized when empty.

Bottles are washed inside and out with hot water and soda solution, rinsed with hot sterile water, and sterilized at 104°C. for thirty minutes. When cool they are filled automatically, stoppered and sealed. Only sterilized glass stoppers are used. The piping system is sterilized daily with steam.

The laboratory work consists in checking methods of operation by frequent sampling of various parts of the system. Also frequent inspections of the different bottling houses are made, and a score card record of the visit is kept. Recording thermometers check the temperature of the sterilizers and these records are kept on file.

Analytical results show a product unchanged in the course of handling.

Bacteria in Commerical, Bottled Waters. MAUD MASON OBST.

The official supervision of commercial, bottled waters has led to the accumulation of a large amount of data concerning their bacterial content. Waters from 167 sources, both foreign and American, have been examined. Many contained large numbers of organisms, including *B. cloacae, paratyphi, mycoides, aerogenes, subtilis, aurantiacus, maritinum, ovale, prodigiosus, fluorescens (liquefaciens), fluorescens, (non-liquefaciens), M. citreus*, long-chain streptococci, and unidentified chromogens. Occasionally, common molds were found, and from one source a sporotrichum occurred in large numbers. A sample from one spring gave cultures of *P. italicum*, and from one import sample were obtained Actinomyces. *B. coli* were isolated from 57 per cent of the domestic samples and from 49 per cent of the import samples in 10 cc. quantities, from 44 per cent of the former and 28 per cent of the latter in 0.1 cc. quantities, and from 9 per cent and 3 per cent, respectively, in 0.001 cc. quantities.

In certain cases, inspections of the springs have located the sources of pollutions in some controllable place, as in the bottles or bottling houses or in a less easily controlled place, as in the spring. When the source of pollution could not be removed, the bottled product was not considered safe for human consumption.

Communications from other bacteriologists have shown that nearly all expect to find bottled waters more nearly bacteriologically pure than municipal supplies, and many feel that bottled waters should at least contain no *B. coli* in more than one of 10 cc. portions.

Comparison of Rapid Method of Counting Bacteria in Milk with Standard Method. W. D. FROST.

The method consists of making small plate cultures, four square centimeters in area, on microscopical glass slides. One twentieth of a

cc. of milk, or less, is mixed with an equal amount of nutrient agar. These "lilliputian" plates are incubated at 37°C. for from three to twelve hours, depending upon the character of the milk. The little plates are then air dried, fixed, treated with ten per cent acetic acid in alcohol, stained in Loeffler's methylene blue (1:4), slightly decolorized in alcohol, and dried. The colonies are stained a deep blue, while the background is a light blue.

The number of colonies in twenty microscopic fields is counted and the number of colonies on the entire plate calculated. This number multiplied by the dilution factor gives the number of bacteria per cubic centimeter of milk. The magnification used should be from 100 to 200 diameters. Results of thirty-seven comparative tests are given. The number of bacteria in these milks varied from 675 to 20,750,000 per cubic centimeter.

The correspondence seems reasonably close. The difference between the two counts usually amounts to less than the differences which occur between duplicate plates or the counts obtained by means of different dilutions in the same analysis, or the counts obtained on the same milk by different analysts.

The preparation of the plates requires less time than the preparation of the standard plates, the staining and counting a trifle more. No expensive apparatus is required. The amount of culture medium is very small. The time required to complete an analysis is never more than twelve hours and in many, if not most, cases can be reduced to four or five hours.

Notes on Brine Pickle Fermentation. C. W. BROWN.

In salting cucumbers there may enter the tank many types of micro-organisms; yet only those that can tolerate 12 to 20 per cent salt are concerned in the normal fermentation. The acidity of new brine is practically zero and increases gradually during two to six weeks to 50 per cent $\frac{N}{10}$ or above—a maximum of 75 to 100 per cent. The principal acids are lactic and acetic in ratio of approximately 2: 1 with traces of propionic, butyric, benzoic. During fermentation gases are evolved; the volume is equal to approximately one-half the volume of the tank and consists chiefly of carbon dioxid—80 to 90 per cent; the samples contained *no* hydrogen, *no* oxygen, a trace of methane and a residual gas, presumably nitrogen. In the samples of brine analyzed alcohol was found in traces only.

The acid bacteria are facultative anaerobes, short rods or cocci arranged chiefly in chains of 2 to 5 members, they produce acid from glucose and lactose, litmus milk after a time is rendered acid but is not loppered. The ability to produce gas is questioned in that there is evidence of the strains isolated producing sufficient gas to saturate or nearly saturate the liquid medium. The gas formed during pickle fermentation is produced largely by yeasts, which can tolerate the high percentage of salt: However, motile short rods—colon type—may be isolated frequently from brine during the first stages of the fermen-

tation and these bacteria produce hydrogen in no less quantity than one part to three parts carbon dioxid.

The scum yeasts or torulae which begin to develop upon the surface of the brine during fermentation and later form a thick scum are acid consumers. In wide mouth bottles plugged with cotton the acidity of a 10-inch column of pickle brine was reduced from 74 per cent $\frac{N}{10}$ to 17 per cent in 45 days at room temperature and to alkaline 12 per cent in less than a year's time. Sterile pickle brine in test tubes inoculated with pure cultures of the scum yeasts was reduced in acidity from 35 per cent $\frac{N}{10}$ to neutral or alkaline within 30 days time at 20°C. The first acid to be consumed is the lactic, leaving the acetic until the last, that is, the ratio changes until the acetic is the predominant or only acid. Tubes of pickle brine agar or even tubes of litmus agar to which sterile commercial lactic acid is added—as much as 200 per cent $\frac{N}{10}$ (1.8 per cent pure acid)—when inoculated with these scum yeasts are rendered neutral or alkaline adjacent to the growth of the yeast within a few days' time. Under similar conditions acetic acid is consumed with difficulty.

The fermentation of brine pickles is an associative action of various microorganisms resulting in (1) the using up of those constituents of the cucumber which may be used readily as microbial food—protein made soluble, sugar changed to acid, etc.—; and (2) in preservation of the depleted cucumbers (brine pickles) in the brine containing the by-products. When the acidity which is a potent factor in preservation is destroyed from the surface of the tank downward by the scum yeasts the brine pickles are liable to decomposition.

Sampling Milk for Bacterial Analysis. ROBERT S. BREED.

In the series of comparative studies on the plate and microscopical methods of counting bacteria in milk which are in progress at the New York Agricultural Experiment Station, some tests of methods of sampling have been carried out. In these tests, comparative counts have been made in order to discover whether samples of milk taken in clean test tubes containing preservatives (formaline or corrosive sublimate) were as satisfactory for use in making microscopical counts as iced samples taken in sterile tubes. The results secured are not sufficient to warrant a positive statement but indicate that samples taken with preservatives are as satisfactory as are the iced samples and much more convenient to handle. When an effort was made to keep the preservative samples for days or weeks, it was discovered that they became less satisfactory, the longer they stood. This was not because the organisms lost their staining power or because of any growth of organisms in the samples but because the bacteria floated to the top with the cream which became compact on standing. Some of them also fell to the bottom. Because of the fact that it was impossible to shake a sample so as to break up both the cream and the sediment perfectly, the counts secured from the samples after standing tended to be lower than they should have been.

Counts made from the cream and sediment of both iced and preservative samples showed that this concentration of the bacteria in the cream and in the sediment occurred in all of the samples. Where no cream was present as in skim milk, the bacteria did not rise to the surface but sedimented in large numbers showing that the reason for their concentration in the cream was because they were buoyed up on the fat drops. In the samples studied, there was a strong tendency for the larger clumps of bacteria to concentrate in the cream, the bacterial groups which occurred in the sediment rarely consisting of more than two individuals.

The Pasteurization of Dairy By-products. ROBERT S. BREED AND W. D. DOTTERRER.

In some work done for the New York State Commission for the Investigation of Bovine Tuberculosis during the summer and fall of 1915 on the pasteurization of whey, it has been found that whey, heated between 140 and 180°F. and allowed to cool slowly in the whey tank, sours with an almost pure lactic acid fermentation due to lactic acid bacilli belonging to the *Bacillus bulgaricus* group. Immediately after heating the numbers of bacteria in the raw whey are reduced from millions to tens of thousands per cubic centimeter. During the 18 to 20 hour period which elapses before the whey is returned to the farmers and during which time it is cooling slowly, there is a rapid growth of the lactic acid bacilli which have survived the heating so that the whey contains from tens to hundreds of millions of these organisms per cubic centimeter as delivered to the farmers. The other types of bacteria present (largely spore forming bacilli) do not increase in number to any marked extent. The acidity of the whey as delivered to the farmers was found to vary from 0.3 to 0.4 per cent calculated as lactic acid.

On the other hand the unheated whey which was examined showed an acidity of 1.2 per cent and contained several million miscellaneous bacteria, one and a half billion lactic acid bacilli and about thirty million yeasts per cubic centimeter. Neither of the latter developed on the agar media and would not have been found if the microscopic method of counting had not been used. No heated whey was found which contained yeasts, a condition which suggested that the improvement in the quality of cheese frequently noted where pasteurization has been adopted has arisen from the elimination of yeasts from the whey tank and so from the farmers' milk cans.

On two successive days at one of the factories, the predominant lactic acid organism in the making vat was found to be a bacillus instead of the more common Streptococcus.

The Effect of Air Pressure on Potable Waters During Storage. W. D. FROST AND FREDA M. BACHMANN.

Steel pressure tanks are in common use for storing water. The question is raised whether or not the effect of the air under pressure in these tanks could be injurious to the contained bacteria, or, in other

words, whether these plants could be depended upon to improve the water if it came from a contaminated well.

Experiments conducted with small quantities of water (2 to 3 liters) held in steel chambers at varying degrees of pressure up to 100 pounds per square inch, and at the temperature of a warm room, showed that the contained bacteria increased very rapidly and to an enormous extent. *B. coli* was not affected by the pressure.

When similar samples were held at the temperature of an ice box, but otherwise under the same conditions, the growth was marked but slow. When water held under similar conditions in the ice chest was partially renewed at intervals of 24 hours by pouring out half the water and putting in fresh, the number of bacteria appeared to remain nearly constant.

An examination of several plants in actual operation showed that the water in these tanks remained practically constant so far as their bacterial content was concerned.

The Bacterial Content of Market Oysters. FRED BERRY.

Eight samples of shell oysters and twenty-one samples of shucked oysters, collected from eighteen different retail markets in Chicago were examined according to the methods recommended by the Committee on Standard Methods of Shell-fish Examination. The first sample was collected October 13 and the last April 27. Additional tests were made to determine whether the use of the combined shell liquor of fifteen oysters, as recommended by Smith, would necessitate a different interpretation from that based on the analysis of five individual oysters, as recommended by the Standard Methods Committee. A few samples of shucked oysters were re-tested after storing in the ice-box for forty-eight hours to determine the character of the bacteria which multiplied most rapidly under such conditions.

Of the eight samples of shell oysters, three contained an excessive number of *B. coli*. These were collected October 13, October 23, and March 2, and had a score of 41,140, and 120, respectively. The other five samples were collected in February and four of these had a score of 0, one a score of 23, and the other a score of 5. The lowest count on the shell oysters was 2600 on a sample collected February 8, and the highest was 7,740,000 bacteria per cubic centimeter on a sample collected March 2nd.

Of the twenty-one samples of shucked oysters, none was free from *B. coli*. The minimum was 1 and the maximum 40,000 *B. coli* per cubic centimeter of oyster liquor. Fifteen of the twenty-one samples contained 100 or more *B. coli* per cubic centimeter. Eight of these fifteen contained 1000 or more *B. coli* per cubic centimeter. The count on shucked oysters varied from a minimum of 140,000 to a maximum of 34,000,000 bacteria per cubic centimeter of oyster liquor.

These results may be summarized as follows:

1. The shell oysters purchased at Chicago during February contained fewer bacteria than those purchased in October and March.

2. The season of the year apparently had little influence on the character of the bacterial content of the bulk oysters, a majority of the samples containing a very large number of bacteria, many of which belonged to the *B. coli* group.

3. No definite correlation existed between the total number of bacteria and the number of gas formers found in the samples.

4. The use of five or fifteen shell oysters for a sample did not materially affect the interpretation as to the sanitary quality of the sample when judged by the U. S. Standards.

5. On the basis of the bacteria developing on plain agar at 20°C., on Endo medium at 37°C., and the presumptive test for *B. coli*, the increase in bacteria in bulk oysters during 48 hours storage in the ice-box cannot be interpreted as being due mainly to an increase in intestinal bacteria.

Normal Fermentation of Sauerkraut. LESTER A. ROUND.

The fermentation of sauerkraut was studied in two factories. In the first factory, microscopic and chemical examinations were made while in the second factory a bacteriological study was also made. The microscopic examination showed that bacteria alone are concerned with the proper fermentation. Wherever air came in contact with the kraut or brine, as at the top of the vat, yeasts grew very rapidly after the first week and produced a heavy foul-smelling scum which rapidly destroyed the acid. Analysis of fresh juice from a vat just being filled showed the presence of five million bacteria, 80 per cent of which were glucose fermenters. The remaining 20 per cent were mainly, if not all, yeasts. The high count was due chiefly to the refilling of tanks which had just been emptied and the walls served as a means of inoculation with acid-producing organism. It was found that in the first 24 hours the plate count would go up to about 100,000,000. During the first week it would go up gradually to 200,000,000 to 300,000,000.

The rate of growth of bacteria and the rapidity of fermentation varied directly with the temperature and were much slower in cold weather than in warm. After reaching a maximum, the number of bacteria gradually decreased until at the end of five weeks there were present between four and ten million viable organisms. Lactose-bile fermenting organisms were found in small numbers at the start. These increased rapidly for the first few days and disappeared rapidly after the kraut showed an acidity of plus 7.0. These organisms probably came from the wagons, forks and shoes of the farmers who brought in the cabbage. Examination of the interior of the cabbage-head showed it to be sterile.

Vats showing abnormal fermentation contained a different class of organisms. A study of such vats indicated that bad fermentations in a properly salted vat were due to the growth of unfavorable organisms during the first few days before the normal acid flora had been able to establish itself and produce sufficient acid to stop decomposition. In the course of normal fermentation there was found to be a slight increase in the temperature.

A Study of the Effect of Spices on the Growth of Certain Organisms. FREDA M. BACHMANN.

A study of the preservative effect of spices in foods was made in order to determine the relative efficiency of the different spices in inhibiting the growth of microorganisms. The molds used for inoculation were the common ones found on spoiled fruits and vegetables, species of Rhizopus, Penicillium, Aspergillus, and Alternaria. Of the bacteria, *B. coli*, *B. subtilis*, and *B. prodigiosus* were studied. A yeast isolated from Fleischman's compressed yeast was also used for inoculation. The molds and yeasts were grown on Thaxter's potato hard agar and the bacteria in the ordinary nutrient agar.

A new method for obtaining a double plate was devised in which the agar without the spice covers one-half of a petri dish and that with spice the opposite half. In this way the organisms may be grown on two kinds of media in one plate. The organisms were grown in such double plates, also in spiced agar slants, and on steamed apples to which varying amounts of spice were added. Besides the study of the effect of ground spices, the alcoholic extracts, the active principles, and the oils were used.

Cinnamic aldehyde is most effective in preventing growth of all the organisms studied. Eugenol and oil of allspice also have a considerable preservative effect. Nutmeg is of little value as a preservative and black pepper and ginger have practically no effect. It was found that there is considerable variation in the sensitiveness of different organisms. Molds were found to be more sensitive than the bacteria and yeast. There is a very considerable difference in the amount of spice necessary to prevent germination of mold spores and the amount necessary to inhibit a growth of the mycelium. The results of this study for the most part confirm those of Hoffman and Evans in their work on spices as preservatives.

SANITARY BACTERIOLOGY

UNDER SUPERVISION OF HENRY ALBERT

Influence of Conditions in the Barn Upon the Germ Content of Milk. M. J. PRUCHA AND H. M. WEETER.

The aim in this study was to measure the collective influence of the barn conditions and operations on the bacterial contamination of milk. Pails were steamed before each milking and the samples of milk for the analysis were taken from individual cows when the pail of milk was brought out from the barn into the adjacent milk room.

The study was conducted from March to July in 1914 and 1915 in three different barns. Barn I was very clean, barn II was not as clean as barn I, and barn III was decidedly dirty. 1710 samples were taken in all. The results are summarized in the following table:

BARN	AVERAGE NUMBER OF BACTERIA OF ALL SAMPLES	
	1914	1915
I	2,288	3,229
II	1,073	873
III	6,604	5,255

Table showing the lowest and the highest counts and the average of fifteen samples from each cow, 1914

BARN I				BARN II				BARN III			
No. of cow	Bacteria per 1 cc.			No. of cow	Bacteria per 1 cc.			No. of cow	Bacteria per 1 cc.		
	Average	Lowest	Highest		Average	Lowest	Highest		Average	Lowest	Highest
174	183	17	532	170	222	27	582	1,034	2,667	307	20,365
135	325	47	1,215	116	265	50	815	1,019	2,718	563	7,285
189	387	50	2,502	166	295	53	800	1,031	3,150	1,088	9,725
167	444	45	1,780	123	329	3	1,078	1,025	4,320	970	22,146
150	506	50	1,457	165	356	45	1,087	1,033	4,598	855	18,520
187	585	247	1,932	159	373	63	920	1,032	4,603	1,390	9,275
171	602	272	1,480	557	603	480	820	1,018	5,324	2,120	13,735
155	613	40	2,560	552	635	63	4,025	1,015	6,414	1,453	12,108
63	657	72	3,257	113	636	100	1,307	1,003	13,120	3,425	39,500
110	665	245	1,300	145	657	52	1,860	1,026	19,092	7,480	63,835
177	758	82	1,783	125	698	50	4,373				
35	723	40	5,705	550	735	30	4,862				
130	751	77	2,760	108	770	31	2,247				
176	826	332	1,323	163	813	11	2,530				
156	763	281	2,117	149	966	32	5,075				
182	833	282	3,425	553	1,045	108	2,012				
179	837	103	2,760	556	1,117	92	4,444				
26	872	140	3,800	117	1,217	70	4,025				
192	888	242	2,182	551	1,258	44	3,250				
73	927	192	3,627	554	1,292	107	4,925				
74	931	97	4,850	137	1,369	433	2,587				
178	925	412	1,686	183	1,613	225	6,612				
186	1,042	347	2,400	131	1,878	232	4,675				
152	1,044	90	8,060	175	2,425	1,362	3,450				
190	1,140	337	6,225	118	2,752	11	12,955				
134	1,164	167	3,395	555	3,588	29	33,000				
154	1,307	192	4,157								
184	1,391	135	6,275								
112	2,010	342	6,900								
191	2,213	320	10,135								
180	2,529	132	28,950								
172	3,874	937	8,505								
188	5,231	67	58,275								
111	6,835	3,095	15,812								
55	35,131	2,255	218,250								
	2,288				1,073				6,604		

Relation of Bacteriology to City Milk Standards. H. A. HARDING.

Standards presuppose something to be measured and measurements presuppose comparison.

Satisfactory city milk standards should furnish a basis for accurately comparing the various milks which may be analyzed from three essential standpoints: (1) food value, (2) freedom from disease germs, (3) cleanliness.

Food value. Bacteriology bears no direct relation to food value. It might bear an indirect one if high term content was accompanied by a lowering of food value. In commercial milk this reduction is not appreciable, except as induced acidity interferes with certain uses of milk.

Freedom from disease germs. Bacteriology has everything to do with this feature, but practically we have no method of determining the presence of such germs and protection must be sought through omnibus methods such as pasteurization. Pasteurization control is mainly through time and temperature.

Cleanliness. Added uncleanliness is probably best measured by bacteriological counts if they are made at the time of infection. However, as soon as the elements of time and temperature enter, such counts no longer indicate the character or extent of contamination.

Conclusion. Quantitative bacterial standards of 1,000,000 or any similar number do not throw any light upon two of the three elements which are important in judging a milk supply, and unless the age and temperature history of the milk is known they do not give any important information regarding the third element.

Purification of Sewage by Aeration in the Presence of Activated Sludge. EDWARD BARTOW.

By blowing air into sewage then allowing the suspended matter to settle and decanting the supernatant liquid, adding fresh sewage and repeating the operation, there is accumulated sludge which has the property of purifying sewage, in the presence of air in from four to five hours. The sludge obtained contains more nitrogen than sludge obtained by any other method of sewage purification. It has been shown by analyses and by experiments with growing plants that it is valuable as a fertilizer. By the process a bacterial reduction of 95 to 99 per cent is effected. The cost of the process depends upon the cost of producing air. It has been estimated that it will be the most effective and most economical method of sewage purification. This will be especially true if the sludge can be readily recovered and disposed of for use as a fertilizer. Plants of considerable size have been constructed at Milwaukee, Cleveland and Champaign and the process will be given a thorough trial.

Diphtheria Diagnosis by means of Blood Serum containing Potassium Tellurate. WILL SHIMER.

The medium for diphtheria cultures devised by Conradi and Troch has not been generally adopted apparently for two reasons; first, the tellurite salt instead of the tellurate salts has been used by most workers, second, the Conradi medium was first recommended as a color differentiating medium as well as an inhibiting. The color differentiation medium is now believed to be of little help.

The Bacteriological Laboratory of the Indiana State Board of

Health has used three dilutions of potassium tellurate: e.g., 1.4, 1.5, and 1.6 cc. of a 1 per cent solution of freshly made up potassium tellurate for each 100 cc. ordinary Loeffler blood serum.

Three hundred and eleven parallel diagnostic cultures on ordinary Loeffler's blood serum and the same number containing 1.6 cc. potassium tellurate per 100 cc. were made. This dilution of potassium tellurate medium gave 2 per cent less positives than the Loeffler's blood serum. Of 246 parallel diagnostic cultures on ordinary Loeffler's blood serum and the same number containing 1.4 cc. potassium tellurate per 100 cc., the dilution of potassium tellurate medium gave 1.2 per cent less positives than did the Loeffler's blood serum. Of 890 parallel diagnostic cultures on ordinary Loeffler's blood serum and the same number containing 1.5 cc. potassium tellurate per 100 cc. the potassium tellurate dilution medium gave 2 per cent more positives than the Loeffler's blood serum.

The increased number of positives obtained with the potassium tellurate medium is not by any means a measure of the complete advantage of this medium. Smears made from the potassium tellurate medium contain fewer bacteria, and their use decreases the time necessary to examine the microscopic slides almost half and lessens the work of getting pure cultures enormously.

The Number of Bacteria in the Air of Cow Stables. G. L. A. RUEHLE.

In the course of an investigation of the air as a source of bacteria in milk which has been made at the New York Agricultural Experiment Station, it was necessary to make a large number of analyses of stable air under a variety of conditions. Altogether 1130 separate analyses of air samples were made but since many of them were duplicate analyses or were made under artificial conditions only 402 analyses are summarized in the work reported upon here. Of these 344 were made in the Station stable and 58 in commercial dairy stables. The aeroscope used in the majority of cases was a simple modification of the sand filter aeroscope recommended by the Committee on Standard Methods for the Examination of Air. The modification was of such a nature as to permit dry sterilization, at the same time eliminating some of the joints where leakage might occur.

The average germ content of the air in 344 tests was 115 per liter. The lowest number of bacteria was found, as would naturally be expected, when the barn was empty and everything was quiet. Sixty analyses made under these conditions showed an average of 41 per liter. The highest average numbers were found after milking was finished and silage was being fed. This caused the cattle to move about, stirring up an evident dust. Ten analyses taken under these conditions showed an average of 271 per liter. Individual tests among the 344 analyses gave results varying from 0 to 825 per liter. The germ content of the air of three commercial stables was found to be similar to that of the Station stable except that four analyses taken

under dusty conditions occasioned by feeding hay or corn stalks gave noticeably higher figures than any of those recorded above. The results of these four analyses were 1100, 2400, 3957 and 16,070 per liter respectively.

From the foregoing results, it is evident that the air of dairy stables contains many more bacteria than have been found by Winslow and Browne (*Monthly Weather Bureau*, 42: 452–453, 1914) in country air, city street air, offices, factories and schools. This is not surprising as relatively dusty operations such as feeding dry hay, grain and the like must be carried out several times daily in every cow stable. In spite of this fact, it must not be concluded that air plays a great part, numerically, in the contamination of milk by bacteria. The studies made in order to discover the relative importance of this factor in milk contamination have shown that the air is a relatively unimportant source of bacteria in milk. The detailed results of the latter investigations are published in *Bulletin 409* of the N. Y. Agr. Exp. Sta. which has just been issued.

Validity of Presumptive Tests. W. F. MONFORT.

Each presumptive coli test proposed from time to time has been first applied locally. Its extension to other regions and other classes of waters has developed certain limitations. None has proved of universal application. It is therefore of first importance that the saving clause of Standard Methods, 1912, page 96, be given due consideration in evaluating any abridged test for the colon group before its adoption with respect to waters of a class or region new to the investigator. This discrimination is not always practiced.

There follow some results of such an evaluation with respect to a surface water (Missouri River) from which a turbidity of 1000 to 12,000 parts per million has been removed; the effluent is treated with bleach. Observations covering more than two years show this supply to contain usually not more than two organisms of the colon group per 100 cubic centimeters.

Neutral red bile-salt lactose broth gives positive results in all dilutions.

Aesculin bile-salt broth, giving negative results with *B. cloacae*, yields a brown coloration with an organism of frequent occurrence belonging to the class *B. fluorescens*.

Lactose-bile gives an error of over 73 per cent as compared with confirmation tests of lactose-fermenting, acid-forming, aerobic bacteria.

In lactose broth 80 per cent of gas formers fail of confirmation.

For a water of this class apparently nothing thus far proposed short of actual discriminatory tests, at least so far as outlined in the lately adopted "standard" for waters used on common carriers in interstate commerce, can be considered valid.

INFECTION AND IMMUNITY

UNDER SUPERVISION OF A. I. KENDALL

A Study of the Bacteria of Normal and Decayed Teeth. I. J. KLIGLER.

Material collected from deposits on teeth of 40 individuals was studied with the object of determining the numbers and types of bacteria found in such deposits, normally and at various stages of decay. Twenty specimens were taken from healthy teeth in mouths of different states of cleanliness, and twenty from carious teeth in different degrees of decay. The complete results of this investigation were published in *The Journal of the Allied Dental Societies*, 1915, vol. x, pp. 141–166, 282–330 and 445–458.

Bacterium pyogenes Associated with a Case of Multiple Arthritis in a Hog. ARCHIBALD R. WARD.

The writer pointed out that polyarthritis of swine is a condition frequently encountered in postmortem inspection of meat. A case showing various stages of articular involvement from the early stages of synovitis to later stages showing erosion of articular cartilage, exostosis and anchylosis of the joints was subjected to bacteriological and pathological examination. *Bacterium pyogenes* was isolated in pure culture by the method suggested by Künnemann. This consists of employing agar to which has been added about 30 per cent of sterile raw cattle serum, just previous to pouring the plates. The organism was also isolated from three abscesses near a joint. The walls of two of these abscesses were in contact with the synovial membrane. The abscesses contained an odorous pus greenish yellow in color.

The synovial membrane was highly reddened and was covered with vegetations in the form of minute vascularized tufts or tassels. Sections of the membrane stained by the Gram method showed organisms similar to *Bacterium pyogenes* within certain cells.

The organism in question has been found by European investigators to be very frequently encountered in chronic suppurative conditions in both cattle and swine, observations that have been confirmed by the present writer.

Spirochaeta Hyos.—Its Antigenic Value in Complement Fixation Tests on Hog Cholera Sera. Studies on Hog Cholera. WALTER E. KING AND R. H. DRAKE.

With antigen prepared from pure cultures of *Spirochaeta hyos*, 115 complement fixation tests have been conducted up to the present time. Of these, 22 tests were with normal hog sera from 10 different animals, 1 from an animal which exhibited a reaction only following inoculation with virus, 6 tests from 2 convalescent or naturally immune swine, 84 tests with sera from 34 animals suffering from hog cholera (4 of which had been used as normals) and one test each with 2 different lots of hyperimmune serum. Negative readings occurred in all cases in which normal hog sera were subjected to complement fixation tests.

sitive readings resulted in all tests with sera from cholera hogs with 2 exceptions.

Complement fixation is coincident with clinical symptoms and depends upon the virulence of the infecting material and the individual resistance of the animal.

Tests of two convalescent hogs indicate that complement binding substances cease to exist in the blood of hogs when immunity against hog cholera becomes fully established.

Control antigens made from cultures of *B. choleras-uis*, *B. Voldagsen* (Haendel), *B. typhi-suis* (Glaesser) fail to exhibit complement fixation with cholera sera.

Antigen prepared from pure cultures of *Spirochaeta hyos* possesses no complement binding properties upon sera of hogs suffering from septicemia, *B. cholera-suis* infection, Anthrax, Ghon-Saohs infection, brine poisoning, or pneumonia from natural exposure.

We believe that, by the observance of proper technique, the results recorded herein can be duplicated without difficulty and that the method may be used to practical advantage as a reliable, accurate means of laboratory diagnosis of hog cholera. Furthermore, the results of these experiments support our former conclusions that *Spirochaeta hyos* merits serious consideration as an organism possessing specific pathogenic properties in relation to hog cholera.

Antigenic Properties of Autolysed Bacteria. GEORGE H. ROBINSON.

Meningococci were allowed to autolyse at different temperatures for varying periods of time. The filtrates and residues were tested for their complement fixing and complement absorbing properties. Only after 24 hours in distilled water at 56° is the fixing power of the filtrate greater than that of the residue. The fixing power of autolysed suspensions decreases in proportion to the extent of autolysis indicating a degradation of the protein. A small portion of the original antigenic substance is obtained in a filtrate after autolysis. A fresh, washed, bacterial suspension gives more satisfactory results as an antigen for complement fixation tests than an autolysate.

The Effect on Horses of Feed Heavily Inoculated with B. coli Isolated from Oat Hay. ROBERT GRAHAM AND L. R. HIMMELBERGER.

The occurrence of *B. coli* or colon-like organisms on grains has been demonstrated numerous times. Recently Rogers, Clark and Evans[1] published a report of their studies of colon bacteria on grains. They isolated one hundred and sixty-six cultures, of which seventy-five were obtained from corn, six from barley, thirty from wheat and forty-one from oats. The grains used were secured from the grain inspection laboratory and should therefore represent average samples grown throughout the different sections of the United States. This widespread occurrence of colon-like organisms on grains, together with the fact that *B. coli* or colon like organisms appeared to be constantly

[1] Journal of Infectious Diseases, vol. 17, no. 1, 1915.

present on oats which were proved to be the cause of a serious outbreak of a disease commonly known as "forage poisoning," suggested the possibility of some pathogenic or virulent type occurring on grains, thus explaining some of the losses occurring to the live stock industry.

To determine the effect produced by ingestion of strains of *B. coli* isolated at this laboratory, horses were fed a wholesome feed heavily inoculated with the isolated cultures of *B. coli* grown on broth and agar media. One horse received agar cultures on corn meal in addition to oats which had previously been inoculated. After four days this animal developed diarrhea, showed a sluggish attitude, and regardless of the amount of wholesome feed consumed lost in weight. A mule was fed 200 cc. of broth culture ranging from forty-eight to seventy-two hours old twice daily. In this animal loss of appetite occurred and the animal became weak and suffered from diarrhea.

A third horse was fed for eighteen days on oats heavily inoculated with *B. coli* after being frozen for four days at 30°F. and allowed to thaw slowly. This animal evidenced an indifferent appetite, was greatly depressed and lost in body weight. Another horse was fed oats which had been previously inoculated with broth cultures of *B. coli*, with the result that the animal suffered loss in weight.

In no case were we able to produce death by feeding, but the condition in the experimental horses was such as to suggest that feeds contaminated extensively with colon bacilli lower animal vitality and render the animal more susceptible to other injury. While most investigators consider colon contamination of grains the result of fertilizing soils with animal fecal matter, some believe that multiplication actually takes place on the grain. For instance, Prescott (cited by Rogers, Clark and Evans) found *B. coli* on grains grown under conditions which gave no history of contamination with fecal material. If this be a tenable view it will account for the occurrence of *B. coli* in greater numbers than can be accounted for by the theory of contamination. Since the toxins of *B. coli* have been proven by Vaughn and Cooley[1] to be intracellular, it follows that the effects observed by us must have been produced by disintegrated bacterial cells. In this connection we desire to mention the effect of daily intravenous injections of dead colon bacilli washed from agar slants. Horses so treated evinced marked symptoms, shortly after treatment, consisting of profuse sweating, uneasiness, increased repiration and exhaustion. In one instance death resulted. In most cases, however, the symptoms subsided in from thirty minutes to four hours after injection, with a noticeable increase in tolerance from day to day.

It is evident from these observations that the occurrence of *B. coli* as isolated from grain which was the causative factor of so-called "forage poisoning" bears no primary relation to the disease resulting from the feeding of the oats, but from a sanitary standpoint it seems advisable to protect animal feeds from *B. coli* contamination in so far as possible.

[1] Journal American Medical Association, 1901.

Further Studies of the Presence of and Significance of Agglutinins for Bact. abortus (Bang) in Cows' Milk. L. H. COOLEDGE.

An application has been made of the complement fixation and agglutination tests using *B. abortus* (Bang) as antigen and replacing the blood serum usually tested with milk. The two tests, when applied to milk from infected udders have checked closely, with the agglutination test a trifle more delicate and reliable. For this reason only the agglutination test is reported in this work.

In every instance where milk direct from the udder was found by animal inoculation or cultural methods to contain *B. abortus* it was also found to agglutinate *B. abortus*. Antibodies were apparently produced locally due to a local *B. abortus* injection as in some instances the milk from only one quarter would be positive while in others all four might be positive with a negative blood reaction. In other instances milk from a quarter would agglutinate the organism when the bacterium could not be demonstrated in the milk by animal inoculation. In these instances the agglutinins may have come from the blood but the indications are that they were produced locally by too slight an infection for the organism to be present in sufficient numbers to cause the disease with the 5 cc. of milk used for inoculation of guinea pigs.

The antibodies usually considered as accompanying infection by this organism have recently been found in the blood of two men and one woman drinking milk from a herd containing infected animals. In two other instances these antibodies appeared in the blood of men drinking milk that was known to be naturally infected with this organism. This method may prove to be another means of safeguarding certified and unpasteurized milk.

This material has been submitted to the *Journal of Agricultural Research*.

The Behavior of Streptococci of Human and Bovine Origin in the Cow's Udder.[1] GEORGE MATHERS.

Bacteriological observations in many epidemics of acute tonsillitis indicate that the causative organism is a virulent hemolytic streptococcus and that the infection is milk-borne. In epidemics in which an infected milk supply is an important factor it becomes necessary to determine the source of the bacteria, and the method by which they gain entrance into the milk. In the instance of epidemic tonsillitis the question naturally arises whether the udder of the suspected cow becomes infected with human streptococci, or whether the organisms causing the outbreak represent bovine streptococci that have suddenly acquired a heightened virulence for man. From a review of the literature it seems probable that hemolytic streptococci derived from bovine sources are of little sanitary significance, and the active factors in

[1] This work was made possible by means of a grant from the Winfield Peck Memorial Fund.

epidemic sore throat are virulent streptococci of human origin. There is still some difference of opinion, however, as to the virulence of these human types of streptococci for the cow. Davis and Capps[2] have reported experiments in which they were able to produce mastitis in cows by the injection of hemolytic streptococci of human origin into the udder, and they have demonstrated conclusively that mastitis may exist in a cow's udder without any physical signs being present other than the invading bacteria and an increased number of leukocytes in the milk. Smith and Brown[3] are inclined to believe from their studies that the streptococci commonly associated with bovine mastitis are different from those found in epidemic sore throat and do not cause human throat infections. Moreover they infer that organisms of human origin do not cause bovine mastitis but may grow and multiply in the milk ducts, a condition which might explain outbreaks of tonsillitis. During the past year an experimental study[4] has been made of the comparative virulence of human and bovine types of streptococci for the cow, along with observations as to the behavior of these organisms over long periods of time in the cow's udder and the following results have been obtained.

In six instances mastitis has been produced in normal milch cows by the injections of small amounts of streptococcus cultures into the milk ducts. Streptococci derived both from human and bovine sources were used in these experiments. It was found that hemolytic streptococci with all the characteristics of the human type may be highly virulent for cows when injected into the milk ducts. They produce a severe mastitis which may result in an atrophy of the mammary gland. It was also observed that organisms of this type may grow and multiply in the milk ducts without causing any visible changes in the udder, but the milk in this instance contained an increased number of leukocytes and streptococci. Hemolytic streptococci from milk and the *Streptococcus lacticus* may produce an acute inflammation of the milk ducts but this change in my experience was of a transitory nature and the mammary gland regained its normal function very rapidly. In these observations streptococci derived from human sources proved to be more virulent for the cow than the milk strains.

In three instances of bovine mastitis all of which were due to hemolytic streptococci of the human type, there were no noteworthy changes in the morphology or cultural characteristics of the invading organisms observed in frequent examinations of the milk throughout the course of the infections. The distinguishing characters primarily noted for each organism are still present, and there are no modifications which might be considered as indicating a change from one type to another. These infections are still active 304, 272 and 234 days respectively after the udders were injected. Also there were no noteworthy changes

[2] Jour. Infec. Dis., 1914, xv, 135.
[3] Jour. Med. Res., 1915, xxxi, 455.
[4] Mathers, G., Jour. Inf. Dis., 1916.

in the distinguishing characteristics of the streptococci of the bovine type during the course of the corresponding experimental udder infections.

It is interesting to note that under experimental conditions the quarters of a cow's udder are apparently separate as regards infection, hence an examination of the milk from each quarter of the udder is necessary before a mastitis can be excluded in the case of a suspected cow.

Bacterial Changes in Uniced Specimens of Water. HENRY ALBERT, JACK J. HINMAN, JR., AND GHARRETT JORDAN.

It is well-known that bacteria tend to multiply rather rapidly in water allowed to remain at ordinary room temperature. The purpose of this investigation was to determine to what extent reliance may be placed on bacteriological examinations of water sent to a distant laboratory.

Examinations of forty different specimens of water of various degrees of purity, were made immediately after collection and again at the end of 8, 24, 48, and 72 hours. The standard methods of the A. P. H. A. were followed.

The following conclusions regarding the bacteriological findings are based on data obtained by a sanitary survey and by both chemical and bacteriological examinations:

1. The usual limit of 100 per cubic centimeter as the total number of bacteria on standard agar plates at 20°C. may fairly apply to uniced samples of water if examined within 8 hours after collection..

2. When uniced specimens of water are not examined until 24 hours after collection, the total number of bacteria at 20°C. which may be permitted in "safe" water may be placed at 200 per cubic centimeter and if not examined until 48 to 72 hours after collection, at 500 per cubic centimeter.

3. The presence of as many as 50 bacteria per cubic centimeter on standard litmus lactose agar at 37°C. should throw suspicion on water examined within 8 hours after collection although a total of 100 may be permitted if not examined until 48 hours after collection.

4. The presence of bacteria producing both acid colonies on standard litmus lactose plates and gas in standard broth throws suspicion on the water as polluted with sewage material, regardless of the length of time that the water has stood after collection.

5. The total number of bacteria in specimens of water which were polluted with sewage material (or probably so) as determined by both a sanitary survey and a chemical analysis is so high that it is not safe to establish limits of bacterial counts.

6. It is possible to depend on the results of bacteriological examinations of uniced specimens of water in a large proportion of cases provided the results are properly interpreted in the light of the sanitary survey, the chemical findings and the bacterial changes that occur in such specimens of water.

Further Studies on the Influence of a Lactose-Containing Diet upon the Intestinal Flora. THOMAS G. HULL AND LEO F. RETTGER.

An ordinary bread and lettuce diet to which is added a considerable amount of lactose will simplify the intestinal flora of the white rat to a single group of organisms—the aciduric group. Milk has the same effect but to a less degree. In rats that have been kept on a high protein diet, *B. Welchii* and *B. coli* are prominent. When lactose is added to this diet the process is much the same as before but slower. All of the Welch bacilli and most of the colon bacilli disappear within five to ten days. The addition of meat to the lactose diet has very little effect if the aciduric flora has been previously established. Milk has but a slight effect upon the meat flora, probably due to the small amount of lactose present.

Three to four hours after feeding a meal containing dry lactose, sugar can be found in suspension for the entire length of the intestine, as well as in the feces. If the lactose is in solution when it is fed, it can be found as far as the ileum. Thus it is seen how lactose, being slowly absorbed, favors the multiplication of the aciduric group.

The reaction of the intestine apparently has little effect upon the flora, the acidity being no greater with the simplified flora than with the mixed flora.

Feeding Experiments with Bacterium pullorum. The Toxicity of Infected Eggs. LEO F. RETTGER, THOMAS G. HULL AND WILLIAM S. STURGES, Yale University.

The problem of eradicating ovarian infection in the domestic fowl must needs assume still greater importance than heretofore, in the light of recently acquired data. Not only is it of the greatest significance to eliminate the permanent carriers of *B. pullorum* from all flocks of fowls from the standpoint of successful poultry breeding, but also because they constitute a possible source of danger to man.

Eggs which harbor *B. pullorum* in the yolk in large numbers may produce abnormal conditions, when fed, not only in young chicks, but in adult fowls, young rabbits, guinea pigs and kittens. The "toxicity" for young rabbits is most pronounced, the infection usually resulting in the death of the animals. In kittens the most prominent symptoms are those of severe food poisoning with members of the para-typhoid group of bacteria. The possibility of infected eggs causing serious disturbances in young children and in the sick and convalescent of all ages must therefore receive due consideration.

Ovarian infection of fowls is very common throughout this country. Hence a large porportion of the marketed eggs must be infected with *B. pullorum*. The latter conclusion is warranted by the fact that of more than 13,000 fowls which were tested by the agglutination method fully ten per cent were positive, and therefore gave unmistakable evidence of infection with the organism in question. When eggs which harbor *B. pullorum* are allowed to remain in nests under broody hens, or in warm storage places, for comparatively few hours, they contain large numbers of the organisms.

Soft-boiling, coddling, and frying on one side only do not necessarily render the yolks free from viable bacteria; therefore, eggs which have gone through such processes may, like raw eggs, be the cause of most serious disturbances at least in persons who are particularly susceptible to such influence, and especially infants.

Studies in Bacterial Nutrition. The Utilization of Proteid and Non-Proteid Nitrogen. LEO F. RETTGER, WILLIAM S. STURGES AND NATHAN BERMAN, Yale Universty.

In a recent publication by Sperry and Rettger it was shown that bacteria are unable to utilize protein nitrogen without the preliminary cleavage of the proteins by enzymes, etc., into their relatively simple products. Further investigations clearly demonstrate that not only unheated (unchanged) proteins resist direct bacterial action, but that purified albumin which has been heated to the point of complete coagulation and sterilization likewise remains unaffected.

It also appears quite certain that albumoses and peptones are not attacked by bacteria, or at the most but feebly, without the aid of a proteolytic enzyme, strong acids, alkali, or extreme heat. Organisms like *B. coli* and *B. typhi* which do not elaborate proteolytic enzymes are unable, therefore, to make free use of albumose and peptone nitrogen. This has been shown in culture tests with weak solutions of both the untreated and partially purified Witte's peptone. For the determination of any possible loss of proteose and peptone, or of albumin, as the case may be, the quantitative biuret method as used and recommended by Vernon has been employed with considerable satisfaction.

What is often regarded as autolysis of *B. coli* and other gelatin-non-liquefying bacteria is not a process of digestion of the protein constituents of the bacterial cells, since there is no reduction in the amount of protein of the medium plus the suspension, and if the protein partially disappears from the cells it is due to agencies other than enzymes, as for example small amounts of acid or alkali, and perhaps mere washing.

A proteose or peptone-digesting enzyme, erepsin, has not been demonstrated in any of the experiments.

Yeasts, Probably Pathogenic, Recovered from Routine Throat Cultures. ARTHUR L. GROVER.

In the past various observers have noted the presence of yeast-like bodies in smears from the throat but no real attempt has been made to study these.

The present investigation covers ninety-cultures showing yeast-like bodies. Fifty-six gave yeast cultures, 3 oidia, 2 leptothrix, 20 gave molds, and 9 gave no fungus. It is interesting to note that the molds and yeasts have identical morphology in the primary smears.

These 56 yeasts could be divided into 17 distinct varieties as shown by the following table:

NO.	DEXTRIN	GLUCOSE	GALACTOSE	LACTOSE	LEVULOSE	MALTOSE	MANNIT	RAFFINOSE	SACCHAROSE	INDOL	INULIN	GLYCERIN	TOP YEAST	APPEARANCE ON SLANT AGAR
1	—	gas*	*	*	*	—	*	*	—	*	—	—	—	Yellowish green heaped up, confluent
2	—	*	—	—	—	—	—	—	—	*	—	*	*	Thin whitish film
3	—	*	*	*	—	—	—	—	—	—	—	—	*	Yellowish green, flat, confluent
4	—	gas*	—	—	gas*	—	—	—	*	—	—	*	*	Bright lemon yellow, heaped up, confluent
5	*	gas*	*	—	—	—	*	*	*	—	—	—	—	At first white, later pink, confluent
6	gas*	gas*	gas*	gas*	gas*	gas*	gas*	—	gas*	—	—	*	*	Like sheets of yellow paint
7	—	*	*	—	*	gas*	*	—	*	*	—	*	—	Waxy white, confluent, raised up
8	gas*	gas*	gas*	gas*	gas*	gas*	gas*	gas*	gas*	gas*	—	*	*	Creamy white, rather flat and dry
9	—	*	*	—	*	*	—	—	—	—	—	*	*	Golden yellow, like sheets of paint
10	—	*	gas*	—	—	—	—	—	gas*	—	—	—	—	Dirty gray, dry film
11	*	—	*	—	—	—	—	—	—	—	—	—	*	White like mass of cream cheese
12	*	*	*	—	*	*	—	—	*	—	—	*	*	Greenish yellow, heaped up
13	*	gas*	*	*	gas*	*	—	—	*	*	—	—	—	At first white, then yellowish, finally fawn colored, confluent
14	—	*	—	—	—	—	—	—	*	—	—	*	*	Salmon pink, confluent
15	—	gas*	*	*	gas*	—	—	—	—	N*	—	—	—	White discrete colonies hirsute
16	*	*	—	—	*	—	—	—	—	*	—	—	*	Gray, thin film.
17	*	*	*	—	*	*	—	*	*	—	—	*	*	Pale yellow discrete colonies

* = Acid or top yeast.
N = Nitrites

Subcutaneous injection into guinea pigs in eleven cases gave a general glandular enlargement. It was possible to recover the yeasts from the glands. Eight of these gave a false membrane in guinea pigs when rubbed on an abraded surface of the mucous membrane lining the cheek. In three cases this membrane extended down over the entire throat. The clinical history of the cases from which the yeasts were recovered in numerous cases showed membranous angina and the absence of the *Bacillus diphtheriae*.

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

BACTERIOLOGY OF FOOD

Effects of Refrigeration Upon the Larvae of Trichinella spiralis. B. H. Ransom. (J. Agr. Res., 1916, *5*, 819–854).

This work was planned to show whether the refrigeration of meat was a safeguard against the spread of trichinosis. Trichinous meat was kept for periods varying from a few minutes up to fifty-seven days at various temperatures below the freezing point of water, and then after gradual thawing was fed to test animals, generally rats. Refrigeration at temperatures as low as 50°C. for twenty days or longer, although not always killing the larvae, so influenced them that the meat could no longer cause infection. A temperature of 41°C. generally killed them in ten days or less. The author concludes that a refrigeration for twenty days at 41°C. may be regarded as always sufficient to render trichinous meat safe for consumption.—H. J. C.

The Bacterial Examination of Sausages and Its Sanitary Significance. W. E. Cary. (Amer. Jour. of Public Health, 1916, *6*, 124–135).

The author found that the bacterial content of sausages bears no relation to the sanitary conditions of the shop. The average count of 16 samples taken from shops scored by the author as insanitary was 24,000 per gram at 37°C. and 2,133,000 at 20°C., while the count of 18 samples collected from sanitary shops was 241,000 per gram at 37°C. and 13,280,000 at 20°C. *B. coli* was found in 94 per cent of the samples. Organisms biologically related to, but not identical with, the enteritidis group were present in 25 per cent of the samples, and *Proteus vulgaris* was found in 33 per cent of them. Starch as an adulterant was detected in 56 per cent of the samples. Skins used as casings, if properly prepared, cannot be considered to increase the bacterial content. Cooking destroyed from 93.3 per cent to 100 per cent of the bacteria present.—D. G.

BACTERIOLOGY OF SOILS

Some Factors Influencing the Longevity of Soil Micro-organisms Subjected to Desiccation, with Special Reference to Soil Solution. Ward Giltner and H. Virginia Langworthy. (J. Agr. Res., 1916, *5*, 927–942.)

It has been observed in the past that bacteria are able to resist drying for longer periods in soil than under other conditions. This has been thought to be due to the retention by the soil of moisture in hygroscopic

form. This cannot be the only factor, however, for the longevity of bacteria in various soils is not proportional to the grain-size and hygroscopic moisture.

Recently Van Suchtelen has succeeded in extracting the soil-solution directly from soil. It was found in the course of the present experiments that if bacteria are suspended in the solution extracted by Van Suchtelen's method from a rich clay loam and are then mixed with sand and dried, they live longer than if suspended in physiological salt solution and then dried under similar conditions. This suggests that the reason why bacteria resist drying longer in a rich clay loam than in sand is not only because of the greater amount of hygroscopic moisture present but because there is something present in the soil-solution of the loam that has a protective influence upon the bacteria. The soil-solution was found by Van Suchtelen to contain a slimy material; and the writers suggest that this might be the substance protecting the bacteria when dried.—H. J. C.

A Comparison of the Acid Production of the B. coli Group Isolated from Various Sources. W. W. Browne (Amer. Jour. of Public Health, 1916, *6*, 39–48).

The author undertook this study to determine the amount of acid production in various carbohydrate solutions by members of the *B. coli* group, as a guide to the recentness or remoteness of pollution of oysters in Narragansett Bay. He found that members of this group isolated from either feces or oysters produced their maximum amount of acid in lactose and glucose when incubated at 37°C. for 24 hours; furthermore that the maximum amount of acid was produced by the end of 24 hours. One series of experiments showed that the largest amount of acid was produced in the monosaccharides and hexites (glucose, levulose, galactose, arabinose, xylose, isodulcite, mannite), less in the disaccharides (lactose, maltose), and least in the trisaccharide (raffinose). That is to say the yield of acid varies inversely as the complexity of the sugar. The author concludes that the members of the *B. coli* group isolated from feces produce more acid in carbohydrate solutions than cultures isolated from oysters, the average differences being very slight, but apparently consistent in all the different fermentable media studied.—D. G.

Relation Between Certain Bacterial Activities in Soils and Their Crop-Producing Power. Percy Edgar Browne. Journal of Agricultural Research 1916, *5*, 855–869.

These experiments as a whole represent a line of investigation in soil bacteriology which it is believed will ultimately place the subject on a more practical basis—a basis which will permit the direct application of the results obtained to the solution of soil-fertility problems. The relations between the bacterial activities studied and the actual crop yields on these plots have proved so striking and so consistent that it was felt that accidental coincidence had been practically elimi-

nated and the results might be considerd to give a strong indication that certain bacterial activities in fields are very closely associated with crop yields. Furthermore, the tentative conclusion presents itself that tests of such bacterial activities in the laboratory may indicate quite accurately the crop-producing power of a soil, or, at least, the relative crop-producing power of several soils. If, further, more exhaustive tests confirm these preliminary observations, it may be possible to secure advance information regarding the crop-producing power of soils by means of laboratory tests of bacterial action in those soils.—S. H. A.

BACTERIOLOGY OF WATER AND SEWAGE

Predicts Federal Control of Stream Pollution. EARLE B. PHELPS. Eng. Record, 1916, *73*, 173–4.

Federal policy needed in the supervision of stream conditions and the necessary administrative body to put it into effect.—F. B.

Air Diffusers Tested at Milwaukee, (Wis.) Sewage Plant. T. CHALKLEY HATTON. Eng. Record, 1916, *73*, 255. Ill. Sec. Amer. W. W. Assn. 1916.

Filtros plate, composed of quartz sand baked, of uniform porosity has given the most satisfactory results. The removal of 90 per cent of suspended matter, 95 per cent bacteria and an effluent stable for 5 days was secured at Milwaukee with the continuous flow tank by using 1.75 cubic feet of air per gallon sewage with 4 hours aeration, 20 per cent activated sludge and from 10 to 15 minute sedimentation. The estimated cost is $4.38 per million gallon excluding engine room and plant attendance, and the cost of disposing of the sludge.—F. B.

DAIRY BACTERIOLOGY

Fermented Milks. L. A. ROGERS. Bulletin 319, U. S. Dept. Agr.

A brief résumé of our present knowledge of this subject. The therapeutic and food value of fermented milk is discussed together with the method of preparation of buttermilk, kefir and yogurt. S. H. A.

The Present Status of the Pasteurization of Milk. S. HENRY AYERS. Bulletin 342. U. S. Dept. Agr.

A summary of our present knowledge of the process of pasteurization. The subjects discussed are: Meaning of the term pasteurization; value of pasteurization; extent of pasteurization in the United States; methods of pasteurization; advantages of low temperature pasteurization; temperatures and methods most suitable for pasteurization; supervision of the process of pasteurization; handling pasteurized milk; cost of pasteurizing milk; bacteria which survive pasteurization; modern theories of pasteurization; and the necessity for pasteurization. —S. H. A.

Agglutination Test as a Means of Studying the Presence of Bacterium abortus in Milk. L. H. COOLEDGE. (J. Agr. Res., 1916, *5*, 871–875).

In testing a large number of samples of milk to determine the presence of the causal organism of contagious abortion, the only previously proposed technique that proved available was animal inoculation—an unsatisfactory procedure because of the length of time required. The writer, has therefore, worked out a method of employing the agglutination test, using 48-hour agar cultures of *B. abortus* as antigen. Negative results by this test always indicated absence of the organism in question; but positive results did not necessarily prove its presence. In making a long series of tests, however, it was found to reduce the number of suspicious cases sufficiently so that the use of animal inoculation was practical in those few cases in which some particular cow's milk did cause agglutination.—H. J. C.

Study of Condensed and Evaporated Milks. IDA A. BENGSTON. Jour. Home Econ., 1916, *8*, 29–33.

The present extensive use of condensed and evaporated milk products makes the proper control of manufacture, and the establishment of standards of purity and food value imperative. Meager work has been done on the bacteriology of these milk products. The methods of preparation of evaporated milk may assure a sterile product. This is not true, however, of condensed milk. The bacteria found are chiefly those that survive pasteurization, and their number may be as high as 1,000,000 per cc. The high sugar content of the condensed milk inhibits the multiplication of many forms.

Streptococci, staphylococci, *B. sporogenes*, lactic acid producing bacilli, *B. subtilis*, *B. mesentericus*, *B. coli*, and yeasts have been found. —C. M. H.

DISINFECTION

Phenol Coefficient of Germicides. F. B. KILMER, A. W. CLARK AND P. HAMPTON. (Jour. Ind. and Eng. Chem., 1916, *8*, 45.

Study of reliability of Hygienic Laboratory method for testing disinfectants. Tests made in two laboratories gave concordant results provided following medium was used: Liebig's extract, 3 grams; salt, 5 grams; Peptone (Witte), 10 grams; water, 1000 cc.; Composition of medium important.—I. J. K.

IMMUNOLOGY

Anaphylatoxin and the Mechanism of Anaphylaxis. RICHARD WEIL. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 37–39.

Precipitin is identical with the antibody effective in passive sensitization. Precipitating antibody heated at 72° for one-half hour lost its capacity to bind complement in the presence of antigen but still retained its sensitizing value as shown by injection into animals. The

conclusion is drawn that complement plays no part in the anaphylactic reaction and therefore that anaphylatoxin plays no rôle in this phenomenon.—W. J. M.

On the Mechanism of Anaphylaxis and Antianaphylaxis. J. Bronfenbrenner. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 19–21.

The author regards anaphylaxis as due to toxic split products of the normal serum proteins produced by the action of the normal tryptic ferment of the blood after the inhibitory influence of the colloids has been diminished by the specific interaction of antigen and antibody. Antianaphylaxis is explained as the result of antitryptic influence of split products of products of proteolysis.—W. J. M.

Agglutination of Bacteria in vivo; Its Relation to the Destruction of Bacteria Within the Infected Host and to Septicaemia. C. F. Bull. Proc. Soc. Biol., and Med., 1915, *13*, 32–33.

Intravenous injection of immune serum causes an abrupt clumping of bacteria in the circulating blood in bacteremia and their accumulation in the internal organs, where they are phagocyted.—W. J. M.

The Utilization of "Reactor" Milk in Tuberculo-medicine. C. B. Fitzpatrick. Proc. Soc. Biol. and Med., 1915, *13*, 35–37.

Cows in excellent physical condition, but reacting to tuberculin, were used. Seven patients with moderately advanced pulmonary tuberculosis were fed upon their milk and showed improvement as compared with control cases on normal milk.—W. J. M.

Late Results in Active Immunization with Diphtheria Toxin-Antitoxin and with Toxin-Antitoxin Combined with Diphtheria Bacilli. W. H. Park and Abraham Zingher. Proc. N. Y. Path. Soc., 1915, N. S. *15*, 110–116.

Individuals giving a negative Schick test before treatment are probably immune for life. Those who give a positive Schick test and who have been exposed to diphtheria should receive either antitoxin alone or, for longer protection, both antitoxin and toxin-antitoxin injections. For general prophylaxis against diphtheria, not including immediate contacts, toxin-antitoxin alone, or with the addition of killed diphtheria bacilli, is recommended. The dose is 1 cc. of toxin-antitoxin (85 to 90 per cent of the L+ dose of toxin to each unit of antitoxin) and 1,000,000,000 bacteria, injected subcutaneously three times at intervals of six or seven days. The early and the late results should be controlled by the Schick test, early results within four weeks and late results from four months to two years after the immunizing injection.—W. J. M.

Agglutination in Pertussis. O. R. Povitzky and E. Worth. Arch. Int. Med. 1916, *17*, 279–292.

The Bordet-Gengou bacillus, grown upon coagulated horse blood veal agar, is readily agglutinated by immune serum. An agglutinating

serum can generally be obtained from rabbits after ten or twelve intraperitoneal injections of living bacilli at seven day intervals. The agglutination test demonstrates the unity of the pertussis group and differentiates this group from the hemoglobinophilic and pertussis-like organisms. In the diagnosis of pertussis a positive agglutination test at a dilution of 1:200 is necessary, in order to eliminate the presence of natural agglutinins.—G. H. R.

Treatment of Typhoid Fever by Intravenous Injections of Polyvalent Sensitized Typhoid Vaccine Sediment. Studies in Typhoid Immunization VI. F. P. Gay and H. T. Chickering. Arch. Int. Med. 1916, *17*, 303–328.

The report deals with the treatment of 53 cases of typhoid fever, the diagnosis being confirmed by laboratory examination, with the sensitized vaccine of Gay and Claypole. The treatment consists of one or more intravenous injections of 1/50 to 1/25 milligram of the vaccine, or, in some cases, an intravenous injection followed by three subcutaneous injections of 1/10 milligram each. The symptoms following the intravenous injections are mild, and the results generally beneficial unless the dose is too large. Of these 53 cases, 66 per cent showed distinct improvement and 34 per cent were relatively unaffected. The curative results are regarded as due to the hyperleukocytosis and the increased amount of antibodies induced by the vaccine. In a few patients having low antibody (agglutinin) titer the vaccine treatment was supplemented by the intravenous injection of considerable amounts of typhoid-immune goat serum. The superiority of sensitized over non-sensitized vaccine is due to the production of a specific hyperleukocytosis.—G. H. R.

The Mechanism of the Abderhalden Reaction with Bacterial Substrates. G. H. Smith and M. W. Cook. Jour. Infect. Diseases 1916, *18*, 14–19.

Bronfenbrenner, working with tissue substrates had arrived at the conclusion that the Abderhalden reaction can be resolved into two distinct sub-phases; (1) sensitization of the substrate by specific elements of the immune serum, resulting in adsorption of antiferments; (2) autodigestion of the serum; he also concluded that only the former of these reactions was specific. The present authors attempt to ascertain whether the same principles apply to the reaction when bacterial instead of tissue substrates are employed. Immune sera were obtained from rabbits immunized to (a) typhoid, (b) paratyphoid A, (c) *Staphylococcus aureus*; also serum from control rabbits. The serum of each rabbit was combined with its homologous substrate and also with the two non-specific substrates. After the serum-substrate contacts, the tubes were centrifuged and the sera dialyzed, and tested by the Ninhydrin method. The substrates were washed and each divided into four parts, to three of which fresh serum from the immunized rabbits was added; the fourth received normal serum. Contact in cold was allowed for 16 hours, after which the tubes were centrifuged,

the serum dialyzed and tested. In the first place, each serum after having been combined with its specific bacterial substrate, reacted positively, the other combinations being negative. In the second place, each substrate that had already been so combined with its specific serum, upon being subsequently combined with the non-specific sera, acted on all of them so as to yield a positive reaction upon dialysis, thus demonstrating that this phase of the reaction is due to autodigestion of the serum and is non-specific. Whether the sensitization of the substrate corresponds with the usual antigen-antibody reaction is a point left for further study.—P. B. H.

LABORATORY TECHNIQUE

On a Colorimetric Method of Adjusting Bacteriological Culture Media to any Optimum Hydrogen ion Concentration. S. H. Hurwitz, K. F. Meyer and Z. Ostenberg. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 24–26.

The indicator is phenolsulphonephthalein 0.01 per cent. The final adjustment is made after sterilization of the medium, with aseptic technic, the readings being made in a specially devised comparator against a standard color solution.—W. J. M.

The Use of Brilliant Green for the Isolation of Typhoid and Paratyphoid Bacilli from Feces. Charles Krumwiede, Jr., Josephine S. Pratt and Helen I. McWilliams. Jour. Infect. Diseases, 1916, *18*, 1–13.

The success of the authors and others in the use of brilliant green broth for the enrichment of typhoid and paratyphoid bacilli in feces led to the attempt to produce a dye agar. After many trials a medium of the following constitution was found to be satisfactory. Extract of beef (Liebig's) 3 gm., Witte's peptone 10 gm., salt 5 gm., agar 15 gm., water 1000 cc. Dissolve in autoclave; the final reaction is set to the Andrade indicator, adding 1 cc. to a 100 cc. bottle of agar; the reaction may be set at time of preparation or (preferably) when used. If the latter, after dissolving, render slightly alkaline to litmus, bottle in 100 cc. amounts and autoclave. Just before use, adjust 0.6 to 0.7 per cent to phenolphthalein (hot titration) then add to each 100 cc. 1 per cent lactose and 0.1 of glucose (25 per cent sterile solutions) and finally the appropriate amount (0.2, 0.3 or 0.4 cc.) of a 0.1 per cent solution of brilliant green. Use about 16 cc. of agar for each plate, allowing them to stand open until agar has cooled. Inoculate as in Endo plates. The method of use is as follows: Rub up in extract broth a large sample of feces (1: 15 by volume.) Place one loop of suspension on a 0.2 cc. and on a 0.3 cc. plate; streak in order given and then on an Endo plate. Place two loops on each of a similar pair of green dye plates; streak in same order and then on Endo plate. Use a heavy platinum wire looped at end. For a direct agglutination test a macroscopic slide method is employed. For fishing, the Russell medium, with 1 per cent Andrade indicator substituted for litmus is employed. As an added precaution

it is recommended that there be inoculated from the original fecal suspension 0.1 cc. into 1 per cent glucose extract broth containing 1: 300,000 of the brilliant green. If slight growth develops on the green plates, Endo agar is inoculated from the broth tubes after 18 hours. In tests made upon carrier and normal stools, and of convalescents prior to discharge it was found that many fecal types were restrained while the typhoid bacilli developed well. In one instance the positive results were increased 36 per cent over Endo plates. The method also proved successful for the isolation of members of the paratyphoid-enteritidis group from feces.—P. B. H.

MEDICAL BACTERIOLOGY

The Effect of Continuous Electric Light in Experimental Arthritis. W. E. Simmonds and J. L. Moore. Arch. Int. Med. 1916, *17*, 78–81.

Exposure to continuous incandescent electric light prevented or rendered less severe experimental streptococcal arthritis in rabbits. When the light treatment was begun after the development of arthritis, treated animals improved, while control animals continued to develop new lesions.—G. H. R.

Lesions Produced in Rabbits by Repeated Intravenous Injections of Living Colon Bacilli. C. H. Bailey. Proc. Soc. Exp. Biol. and Med., 1915, *13*, 62–63.

Colon bacilli were injected intravenously into rabbits at 3–4 day intervals over long periods. Animals surviving 88 to 142 days showed fibrous and hyaline changes in the kidneys, spleen and liver. In the spleen a material resembling amyloid was formed about the Malpighian bodies but the amyloid nature of this substance was not conclusively demonstrated.—W. J. M.

Tuberculosis in Infancy. C. H. Dunn, Amer. Jour. Diseases of Children, 1916, *11*, 85–94.

The author briefly reviews the various opinions that have been held concerning the portal of entry and the type of the organism in tuberculosis of children. The observations recorded consist of twenty-five autopsies upon infants under two years of age. The examinations were particularly directed toward the lungs and intestines, which were cut into small pieces and all suspicious portions sectioned and examined microscopically. In twenty-two of the twenty-five cases there was found what was regarded as the primary focus and portal of entry. The author therefore, disagrees with the opinion that the tubercle bacillus may in many cases enter the body and leave no local histological evidence. In twenty of the cases the supposed primary was located in the lung and in two it was found in the intestine. In only five cases were animal inoculations made and the type of organism studied. Four of these proved to be human and one bovine. The one bovine culture came from one of the cases in which the primary lesion was located in the intestine.—R. M. T.

The Bacterial Flora of Infected Gun Shot Wounds.—Louis A. LaGarde. The Military Surgeon—1916, *38*, 1–6.

This article is written for the benefit of the military surgeon rather than for the bacteriologist, but reviews some of the bacteriological work that has been done on wounds in the present war. Thus Fleming examined 127 wounds and found that the *B. Welchii* was present in 103, *Bacillus tetanus* in 22, and streptococci in 102 during the first week. Gudgeon, Gardner, and Bawtree found that of 100 wounds all were infected, 99 with various combinations of aerobic and anaerobic bacteria, and one with a pure culture of *B. Welchii*. The article points out that with regard to the bacteriology of gun shot wounds, investigation during the present world war has so far resulted in no new bacteriological data.—E. B. V.

Practical Points in the Prevention of Asiatic Cholera. Allan J. McLaughlin, The Military Surgeon, 1916, *38*, 22–29.

McLaughlin quotes literature showing that presumably healthy individuals have been proven to harbor cholera vibrios in dejecta for periods ranging from 10 days to 69 days, and that Gaffky reported a case who was a carrier for 6 months. The carrier question has therefore become one of the most important factors in any endeavor to stamp out cholera or prevent the entrance of the disease. These long time carriers make a farce of the ordinary 5 day quarantine detention without stool examination. Instead of this, the present method is to examine the stools of all contacts or suspects. In view of the fact that prompt diagnosis is essential, and to avoid time consuming manipulations where large numbers of people are to be examined, the following simple method is recommended: Plate on agar after primary inoculation in peptone enriching media, and test individual suspicious colonies by a macroscopic agglutination on a glass slide, using a very powerful cholera immune serum, which will agglutinate cholera in dilution of 1–4000. This serum may be used in dilution of 1–200 and in this strength will give prompt agglutination with cholera but not with other organisms. Goldberger's enriching solutions, an alkaline egg peptone and on alkaline meat infusion peptone are mentioned with the statement that laboratory tests indicate that they restrain the growth of ordinary faecal bacteria while promoting the growth of cholera vibrios, but that these media have not yet been tested in actual field work.—E. B. V.

PHYSIOLOGY OF BACTERIA

Effect of Natural Low Temperature on Certain Fungi and Bacteria. H. E. Bartram. (J. Agr. Res., 1916, *5*, 651–655.)

Dried cultures of certain molds, Actinomycetes, and bacteria proper were exposed to outdoor conditions at temperatures sometimes as low as −30°C. More than half of the molds survived for four months under these conditions, but most of the bacteria died. Control cul-

tures in the laboratory did not die. Writer does not state whether the cultures kept outdoors were exposed to sunlight as well as to cold.—H. J. C.

Effect of Elemental Sulphur and of Calcium Sulphate on Certain of the Higher and Lower Forms of Plant Life. WALTER PITZ. (J. Agr. Res., 1916, *5*, 771–780.)

These experiments were planned because there has been some disagreement in the past as to whether sulphur compounds increase or decrease plant growth. Tests were made to observe the effect of elemental sulphur and of calcium sulphate upon: (1) total number of bacteria in soil (determined by plate method), (2) growth of pure cultures of the organism causing red clover nodules, (3) accumulation of nitrates and ammonia in soil, (4) growth of clover in soil and in agar culture. The results indicate that elemental sulphur slightly stimulates the growth of red clover, but has a harmful effect upon all the other activities investigated; that calcium sulphate increases the growth of the legume organism and the growth of clover, but has no influence upon the general soil bacterial flora.—H. J. C.

The Action of Schumann Rays on Living Organisms. W. T. BOVIE. Bot. Gaz., 1916, *61*, 1–29.

The source of light was a hydrogen discharge tube, the top of which was closed by a transparent fluorite plate through which the Schumann rays were emitted. In general a small organism was killed more quickly than a large one. The organisms used were rotifers, amoebae, infusoria, Spirogyra and fungus swarm spores. By a number of methods it was shown that the action of the light is on the organism directly and not indirectly by the formation of a toxic substance in the medium. The extreme destructive action of these rays is the result of strong absorption. Because of this absorption, the Schumann rays have a marked localized action, which gives them a peculiar value for investigations in the morphology and physiology of the cell. The change produced is often one which results in an alteration of the equilibrium of the water content of the protoplasm. In the Schumann region of the spectrum, as in the region of longer wave length, the destructive action of the light increases as the wave length decreases, and the light of the Schumann region is much more destructive than the light of the region of longer wave length.—J. T. E.

PLANT PATHOLOGY

A Serious Disease in Forest Nurseries Caused by Peridermium filamentosum. JAMES R. WEIR AND ERNEST E. HUBERT. Jour. Agr. Res., 1916, *5*, 781–785.

Peridermium filamentosum Peck has been found to cause a serious disease of yellow pine seedlings at the Savenac nursery located at Haugan, Mont. The fact that the same species of Peridermium at-

tacks both the lodgepole pine and the yellow pine increases the difficulty of control of this fungus.—S. H. A.

Sweet Potato Scurf. L. L. HARTER. Jour. Agr. Res., 1916, *5*, 787–793.

The scurf disease of the sweet potato was first recognized in 1890 by Halsted, who named the fungus "*Monilochaetes infuscans*," a new genus and species. He failed, however, to describe either the genus or species. The scurf has been found prevalent in nine States and sparingly in others, and on 16 varieties of sweet potatoes. The organism has been shown by inoculation experiments to be the true cause of the disease. A detailed discussion of the morphology of the organism is taken up, also its growth on different culture media at different temperatures. It was found that the organism on the host consisted merely of sporophores and conidia. In the culture, however, well-defined branched mycelia and spores developed.—S. H. A.

Further Studies on Peanut Leafspot. FREDERICK A. WOLF. Jour. Agr. Res., 1916, *5*, 891–902.

A continuation of work on the fungus diseases of peanuts, the object being to secure information regarding the agencies concerned in the distribution of leafspot, *Cercospora personata* (B. and C.) Ellis, and to correlate the destructiveness of the disease with the presence of certain climatic conditions. Crop rotation was not found effective under field conditions in eliminating leafspot; nor was the disease prevented by seed disinfection with copper sulphate or formaldehyde before planting. No correlations between the presence of certain conditions of temperature and moisture and the presence of leafspot exist because of the fact that air currents and certain insects are carriers of *Cercospora personata*.—S. H. A.

Soil Stain, or Scurf of the Sweet Potato. L. J. TAUBENHAUS. Jour. Agr. Res., 1916, *5*, 995–1001.

The economic importance of the disease is discussed, also the occurrence of soil stain, symptoms of soil stain, effect of the disease on the host, factors favorable to soil stain development, the cause of soil stain or scurf and the morphology and physiology of the fungus causing the scurf. The fungus *Monilochaetes infuscans* was found to be difficult to culture because it is a very slow grower and is readily overrun by associated saprophytes. The conidiophores of *M. infuscans* are distinct from the mycelium, the older growth of which is also dark. The conidia are borne in chains which readily break up when moistened or disturbed.—S. H. A.

Factors Involved in the Growth and the Pycnidium Formation of Plenodomus fuscomaculans. GEORGE HERBERT COONS. Jour. Agr. Res. 1916, *5*, 713–770.

This paper gives the result of experiments performed with *Plenodomus fuscomaculans*, a fungus pathogenic to the apple. The organ-

ism was found to have a wider range of conditions suitable for growth than for reproduction. The quantity of food stuffs necessary for growth is extremely minute. Pycnidium production requires more food, but the meager amount present in distilled water is sufficient to allow the production of a few pycnidia. Magnesium sulphate and potassium dihydrogen phosphate in very dilute solutions furnish the necessary mineral elements for growth and reproduction. The carbon supply may be taken from a wide range of compounds of alcoholic structure. Carbohydrates furnish food material in the most available form, and of these xylose and maltose produce the best growth. The nitrogen assimulation is greatly influenced by the type of carbon nutrition. The influence of physical conditions on growth and reproduction is also shown. The general problem of the paper was to study the effect of environmental factors upon *Plenodomus fuscomaculans* especially as they influenced growth and reproduction.—S. H. A.